

Preparation of diploid moults

676

February 4, 1950

D/0 liquid + 0.1% lactose

1-4 single colonies from old 715 bac

5,6 from broad streaks. Asorbate 5 acetate or shalony.

Stock out initially and after growth. Estimate % bac.

Initial	20 h.
25	85
<10	90
40	90
80	>90
50	>90
40	>90

This appears to be a satisfactory method for preparing diploid moults!

Use (4) for irradiation study in hope that high proportion of v is maintained

~~676~~
Irradiation of M226

677

February 5, 1950.

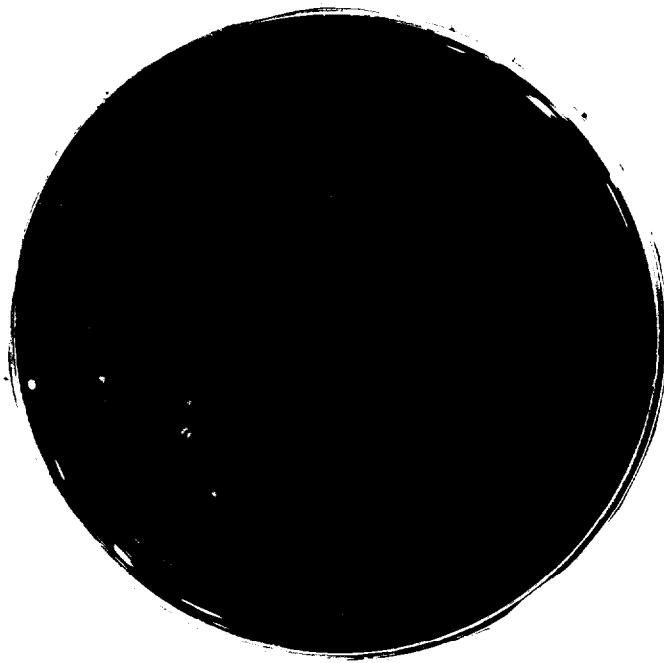
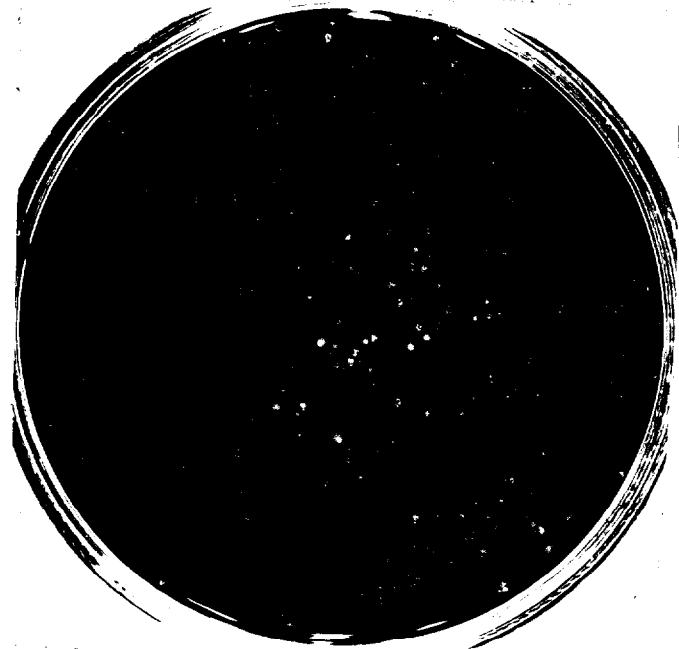
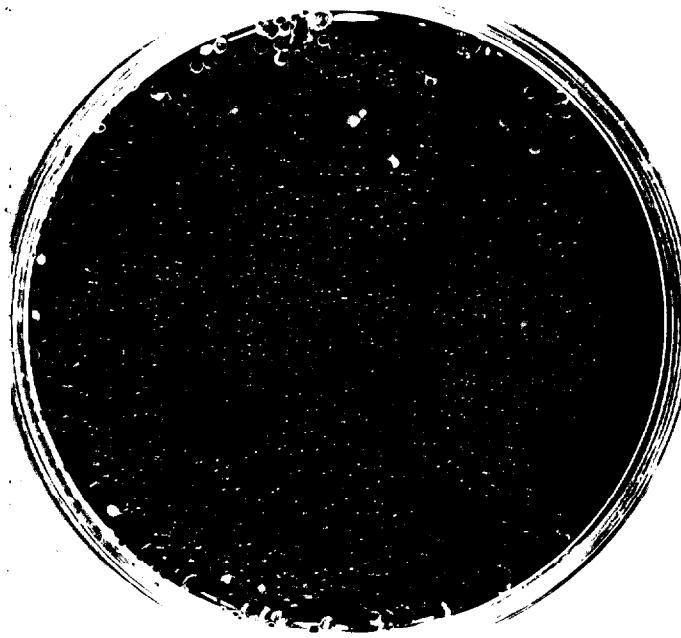
Use liquid culture 676-4, ca 20 hours. O.D. 4.00

Assume titer of ~~10⁹~~, and work for 100 colonies/plate.

For safety, also plate at 10⁻⁶ and 10⁻⁵.

Dilute to ~~10⁻⁴~~. Irradiate 3ml 2secs 20sec low pressure uv.

- A) Take .1 ml samples crowded.
- B) Dilute to ~~10⁻⁴~~ 10⁻⁵ .1 ml sample
- C) 10⁻⁶ .1 ml samples.
- D) Expose *1ml original sample to 57°C. 10 mins.
(.7ml)
Dilute 1:10 in cold H₂O and plate out at various dilutions
All plates sterile.
- E) Plate original sample at all dilutions from 8 - 3.
- | 10 ⁻⁸ | 4 | 0 |
|------------------|-----|------|
| 10 ⁻⁷ | 48 | 4 |
| 10 ⁻⁶ | 305 | 24 |
| 357 | 28 | 385. |
- $5 \times 10^8 = \text{titer}$
- %v.



Count B series.B (no.
uv)

bac

	V	-	Σ
1	170	16	
2	174	10	
3	200	16	
4	197	14	
wip. form	2	0	
	<u>743</u>	<u>56</u>	
per ml	<u>1857</u>	<u>140</u>	<u>1997</u>

Bx (uv
2sec.)

(wip.)

per ml

6	63	
12	64	
0	2	
18	127	
90	<u>635</u>	<u>725</u>

$$\text{Survival} = \frac{725}{1997} = 36\%$$

Shift bac_{uv} from 93 to 12%.

Photograph
sample plates of
Band A, (o, x.)

C now.

per ml.

17	3	
17	1	
9	2	
12	5	
19	2	
1	0	
75	13	
<u>150</u>	<u>26</u>	<u>176</u>
10	65	75
0	9	
1	3	
2	5	
1	9	
4	26	

ex

EMB Mal counts.

Most Mal plates were contaminated.
Some, however, were as clear as clear batch.

B: not readily scored; however, mostly Mal+ or Mal_v.

B_x: Plates contaminated, but fairly numerous scattered colonies:

Mal+(?) Mal- Mal v

34 50 20

The frequency of Mal_v seems higher than of bact., suggesting some
disequilibrium. See 674.

In B, a fair proportion of crescentic colonies was seen (F) (C) etc.

(On E5) a single colony was noted that, by darker appearance, might
be a bact.-recombinant. Check out for chlks. —

Lact+ pure, Mal+, Xyl+, ~~H~~ MtL+. Sug on agar slant.

2/10/50.

A number of colonies previously scored as Lac-; left out on desktop several days, now show central papillae. Pick and streak out on (plate photographed as 677-UV B). EMB Lac.

v - central +
8 29 23

In general, they seem to give typical Lac+!

To avoid any confusion, a special uv experiment is called for!

Treatment of H226 with Mustard (HN2)

678

Febr. 6, 1950.

H226, suspension 676-4 (24h. in refriger.)

A) Control: Titrate out from buffer below.

B) Add 2 ml suspension to ~~1 ml~~ + ml ~~H2O~~ buffer ~~pH 7.0~~10 ml D(Lac) Eastbuffer I. Add 10 mg HN2. After 5 mins.,
dilute 1:10 in Y2 glue to inactivate HN2. Titrate. (Initial 10^{-2})
Record in terms of initial ~~to~~ suspension.

A)	10^{-7}	V,+ 56	- 2	
	10^{-6}	420	22 / 442	
B	10^{-6}	46	206 / 252	

The survivor time appears generally to be ~~base~~ -

Treatment of H226 with chemicals

679

February 6, 1950.

Use suspensions 676-4 and 676-4A (O.D. = 750) $\eta_{Hod} = 150$ 676-4A is loop transfer from D(Lac) to D(Lac).

- A) Assay 4
- B) Assay 4A
- C) Dilute 4 1:5 in 6% sodium deoxycholate. 37° . 52° - 75° p.m. Extract
- D) Dilute 4A 1:5 " " "
- E) Dilute 4A 1:10 in D(~~—~~). Add 10 mg HN2, hydrochloride, (Room temperature)
- F) Dilute 4A 2:10 in 1.2% Methyl Green. $\{ 8^{\circ} \text{ p.m.} - 955 \text{ p.m.} \}$ $10 \text{ min. dilute } 1:10 \text{ in } \text{Pernicious}$
G) " 2:10 in 1.2% " " 37° . 145° exposure

Record debris subsequent to treatments. E as original, cf. B.

H. Suspension 4. Heat to 68° 5 mins..

- I. " " " 10 mins.
- J. " " " 20 mins.

F, G. appear all dead!

UV; HN2; Kill by a nuclear mechanism.
Heat; doch; " " a non-nuclear " ??

579a

A.	dil	V	-
Assay	6	393	45
	7	33	2
B	assay	7	57
	6	n.c.	26
C	doca.	6	72
D	doca.	5	311
		6	9
E	Huillard	5	212
		4	37
F,G	1,...	sterile	
H	4	200	22
I	2	73	5
J	1	9	4
		0	0

Reinfectate!

v much more numerous after after incubation

Office Memorandum • UNITED STATES GOVERNMENT

TO :

226

J-5-50

DATE:

FROM :

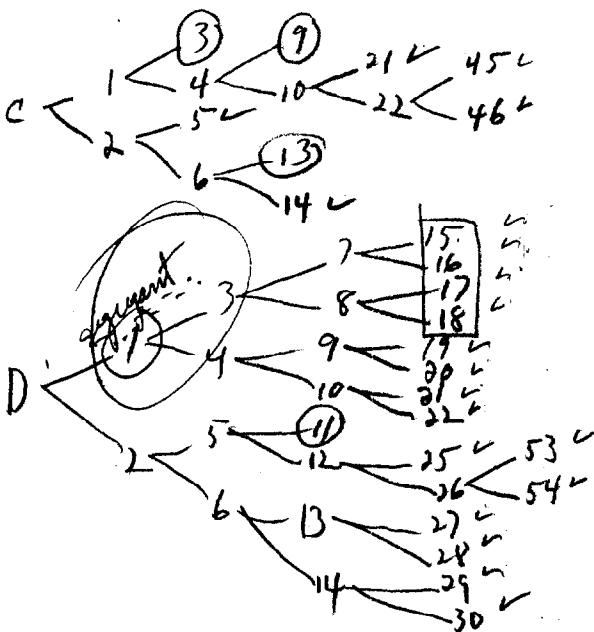
SUBJECT:

Odebutgnew

vacuum relationships

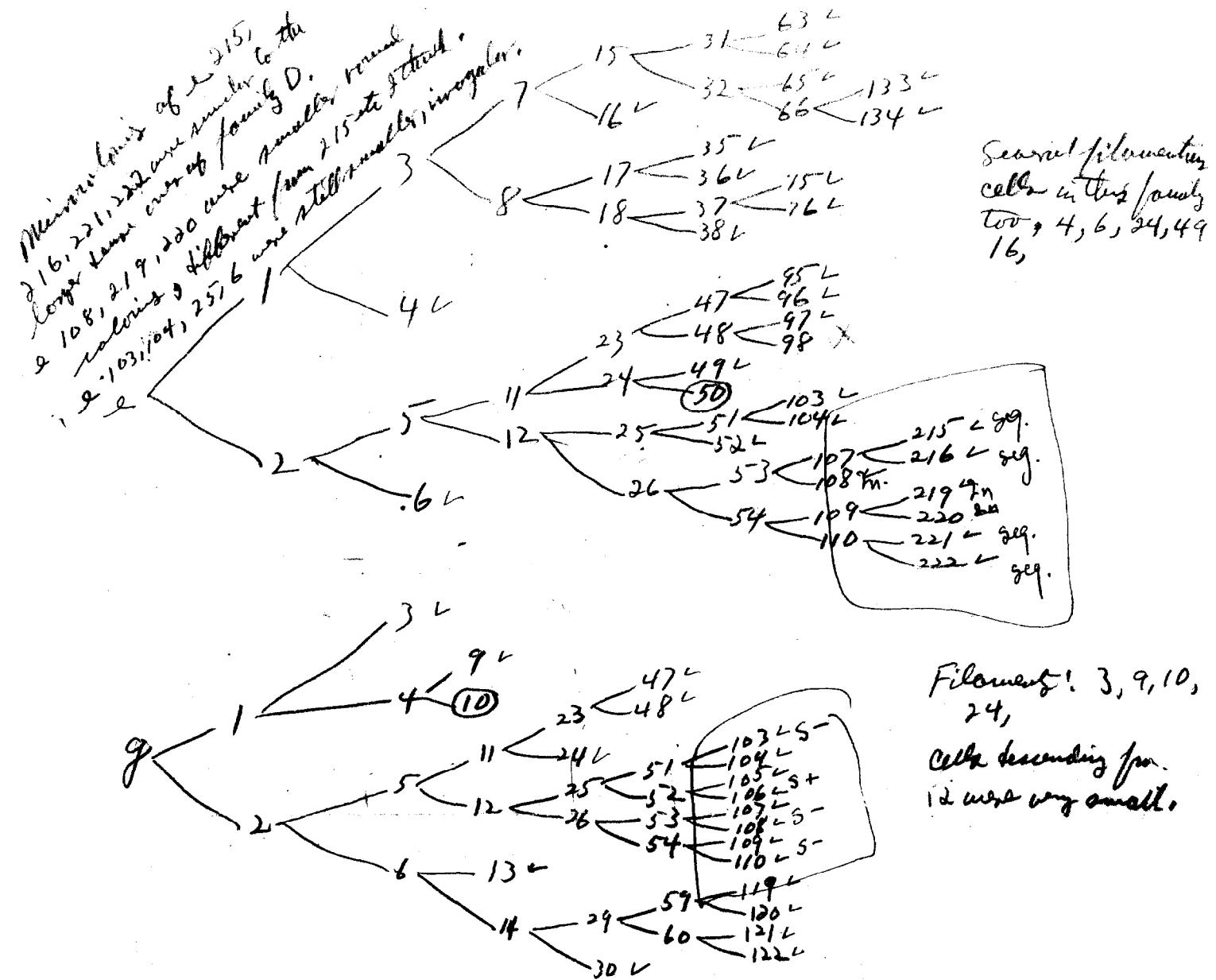
B

This pedigree characterized by very small cells
except #4 which was filamentous + later divided. #2 became a
8-10 μ filament - stopped there.



Most cells in this pedigree
formed filaments, then divided
somewhat irregularly.

Microcolonies of this family
were rather fast growing, dense,
hardly to get into pipette as if
they were mucoid.



Filament! 3, 9, 10,
 24,
 Cells descending from
 12 were very small.

Microcolonies of 105 + 106 were found + smaller than 103, 104 and 107-110.
 107-110 are the dense ones like the D family. 105 + 106 microcolonies were
 similar to e 108, 219, 200.

Zelle: single cell pedigrees on H226

280

February 5, 1950

A.

15 11
33 15
31 25
34 33
77 35
78 36
37 37
53 53
54 54
59 59
60 60
61 61
62 62
77 77
78 78

	Lac	Mal	lacEMS
15	+	+	-
33	-	-	+
31	+	+	+
34	+	+	+
77	+	+	+
78	+	+	+
37	+	+	+
53	+	+	+
54	+	+	+
59	+	+	+
60	+	+	+
61	+	+	+
62	+	+	+
77	+	+	+
78	+	+	+

B

4
33
34
35
36
37
63
64
65
66
77
315
633
634
635
636
637
638

All lac+
Mal+
n.g. no EMS.

started with a segregant

14
21
45
46

680

6

D

三

19

13

134

21

2

2

2

2

EN 81
+ + + + +
Fayalite

1

680

lac

3	+
9	-
13	+
24	-
30	+
47	+
48	+
103	-
105	-
106	-
107	-
108	-
109	-
110	-
119	+
120	+
121	+
122	+

Mal

+++-||-+---+-+++-+++-.

Test segregants.

H226

6802

2/8/50.

V₁

Lac

Mal

Gal

Ar

Xyl

Mte

Stl

Natr.

H226

G
103
104
105
106
107
108
109
1101 R
2 R
3 R
4 R
5 R
6 R
7 R
8 R—
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+TB,
TB,
B,
B,
TB,
TB,
TLB,
TB,pure +
or
gal; ar;
stl???
No M-!

Chemical treatments with H226

681

Febr. 8, 1950.

brothum prepared from EHS colony into S(Lec); incub. 20 h.

$\text{O.D.}_{420\text{m}} = 830$

A. Assay

- B. Dilute 1/10 in .01% Methyl gum ^{in D(-)} ~~215 pm - 545~~
- C. Dilute 1:5 in 6% Na deoxycholate. " "
 Add H₂O to 1/10 Express dilutions as original.

A7.	V 135	— 12
B6	325	37
C6	>>600	ca 10%

Methyl gum : v little killing
no haploidization

Not enough killing!

no haploidization

Treatment of H226 with chemicals.

682

Febr. 9, 1958

Use same H226 susp. as 681.

A) Assay

- B) Dilute 1:50 in 6% Na deoxycholate 11.45° }
C) Dilute 1/10 in 0.1% Methyl Green 11.45° }
D) " " 1/10 in H₂O saline } 37°
[I'm probably in error 10x dil.]

Express as oxi. conc.

	V	-
B6	230	16
C2	114	5
D"7" (8) (2 plates)	13.6 (in) 19	(1,2) 3 3

No appreciable killing in deoxycholate! pH of 6% solution: 8.9!

1:5 bacteria / doca
bacteria found (L.) : 7.1

Methyl Green kills by non-nuclear mechanism.

Chemical Treatments of H226

683

Preliminary Data

February 10, 1950.

Add

~~Take stock of H226 (26 hr. in 50 ml 0.1M) at 1:10 $\xrightarrow{\text{to culture}}$ D(-), plus~~
~~no supplement added. Disinfect in tank unless indicated.~~

		Susp. vol.	Total Vol.
A.	—	0	11.
* B.	Aciflazine .05% dark	0.5	11.5
* C	" " under 4W fluorescent lamp	0.5	11.5
* D.	Pyronin Y .01%	0.1	11.1
E.	NaCl NO 1%	(NaCl NO 5%) 2.5	13.5
F.	hydroquinone 1%	(Hg 5%) 2.5	13.5
G.	Farnaldehyde .04% (= .1% formalin)	1	12.0

530

D ~~B~~ — in H₂O to prevent pts, which is heavy in B- alone.

* 630

B+C agglutinated heavy ppt in D.

930

1 standard loopful, spread on 1 plate serially.

All but A are sterile

Repeat 2/11/50 under less drastic conditions

February 16, 1950.

Same stocks as 683 (refrig.)

		Susp %	vol.	Total
A	-	-	-	last.
B	Acriflavin .005 %	1	.05	11.05
C	" (light)	1	.05	11.05
D	Pyroneine Y .001 %	1	.01	11.01
E	NaClO 0.1 %	5	.2	11.2
F	Hydroquinone 0.1 %	5	.2	11.2
G	Formaldehyde .01% (= .25% formalin) #1	1	.1	11.1

4PM - mix.

Assay at 5PM. A + D

Assays at 10^{-2} ; 10^{-4} ; 10^{-6}

E - G 6PM.

A	7:	ca 300	90% lacv	
B	6:	ca 100	mostly lacv	{ Many colonies \oplus
C	6:	ca 100	" lacv	
D	4:	ca 200	mostly lacv	
E	6:	>500	lacv	No sign. killing ??
F		sterile		
G	6	ca 100	80% lac-	!

∴ formalin has same mode of action as UV; mustard. Pyronine; acriflavin do not, but check for ^{balance} lethal. Hydroquinone is extremely bactericidal.

UV Killing Curve

685

2/13/50.

50 cm; 5 ml samples of H226, diluted 1:100. (H226 is grown culture in flasks of D(8), refrigerated 2-3 days. (See 683). Initial assay est. ca 3×10^6 . After dilution, assume 3×10^7 .

State dilutions as of

uv	D ₁	1:100 sample Count (cells)	Survival	PS
A 0	5	70,61	6.5×10^6	1.0 0
B 10	5	28	3×10^6	0.46 .34
C 20	4	144	1.4×10^6	.21 .68
D 30	4	84	$.8 \times 10^6$.12 .92
E 40	4	25	$.25 \times 10^6$.038 1.38
F 60	3	176	$.18 \times 10^6$.028 1.57
G 80	1	22	2.2×10^5	3.4×10^{-6} 5.
H 100	1	5	5	$.77 \times 10^{-6}$
I 120	1	7	See	1.8×10^{-6}
J 150	1	1	684A.	$.15 \times 10^{-6}$

from 1:10 diln
in dilution
gradient

K Formaldehyde 25% 10 min.
L Hydroquinone .25% ca 12 min.

Assay	60;	8		
K5	68;	7;		
	v	-		
K5	31	99	130	1.3×10^8
L5	461	98	559	5.6×10^8

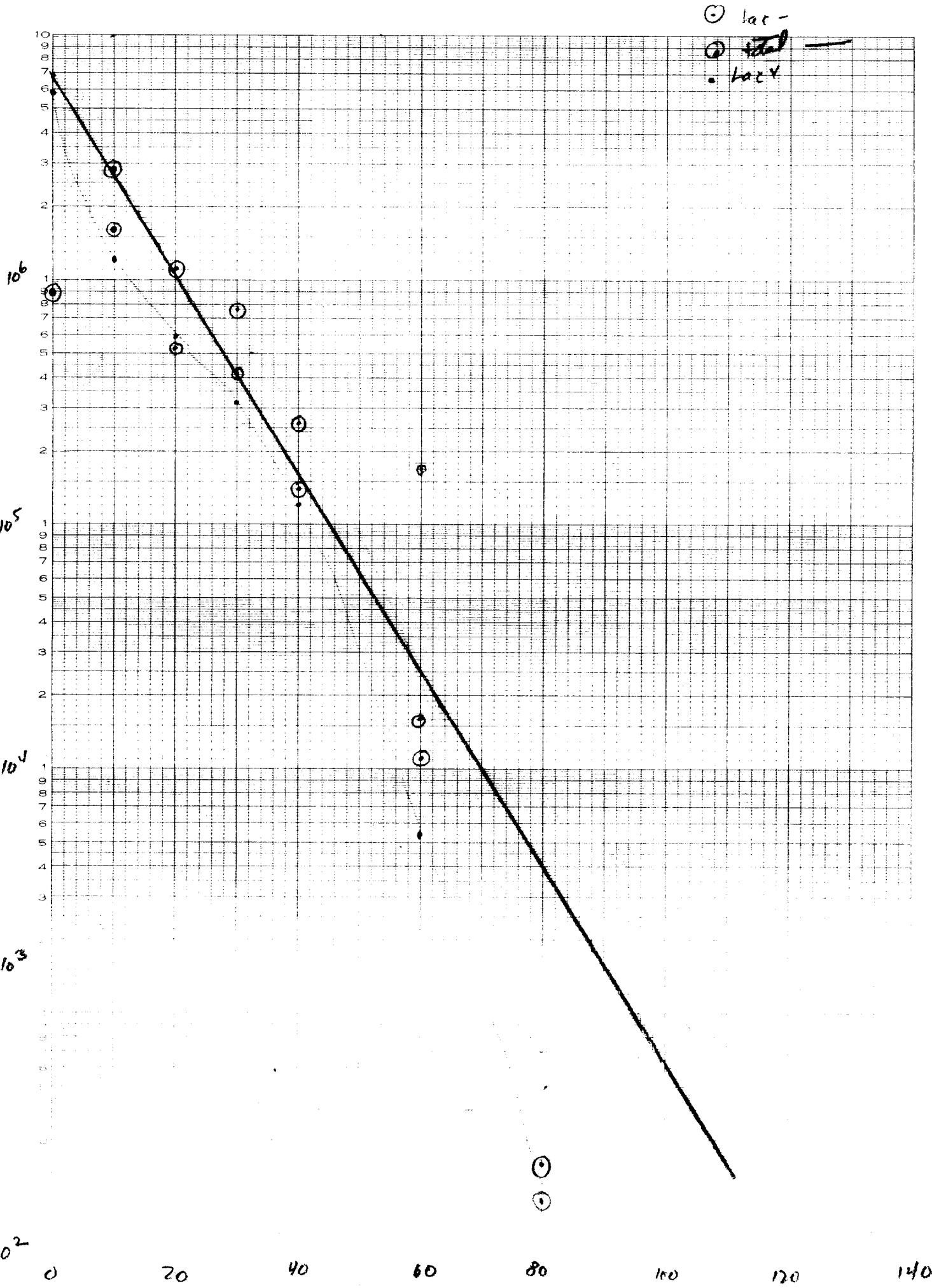
20% survival
Negligible killing,
Inconclusive

68Ya

Survival

	Dil	lacv	lac-	Σ	Σ	v	-	% v
	uv							
A	0	5	54 64 118	10 8 18	64 72 136	3 68	6.8 ⁶ 5.9 ⁶	9 5 ⁵ 87
B	10	5	12	16	28	2.8 ⁶	1.2 ⁶	1.6 ⁶ 43
C	20	4	59	51	110	1.1 ⁶	5.9 ⁵	6.1 ⁵ 54
D	30	4	31	42	73	7.3 ⁵	3.1 ⁵	4.2 ⁵ 42
E	40	4	12	14	26	2.6 ⁵	1.2 ⁵	1.4 ⁵ 46
F	60	"3" "2"	57 51	112 105	161 106	1.7 ⁵ 1.6 ⁴	5.7 ⁴ 5.1 ³	7.1 ⁵ 3.3
G	80	1	7	17	24	240	70	170 29
H	100	1	3	1	4	40	30	10
I	120	1	1	8	9	90		
J	150	1	0	1	1			

Note that proportion of lacv does not vanish, and may reach a minimum with low doses.



February 10, 1950.

Baculum 246. (after 3 h) in D(Lac) (Very dense -- 1.072×10^9)

Plate out — Dilute 1:100; irradiate uv 20 seconds, and plate out.

A) assay 5×10^{-8}

B) Irradiate and assay.

1 10^{-7}
2 10^{-6}
3 10^{-5}

{ Sterile

~~Repeat 2/11/1950 with same suspensions.~~

2/13/50

A. Assay & irradiation.

B. Dilute 1:200 and irradiate 20 sec at 20cm.

4 survivors at 10^{-1} !

too high kill

Express as 1:200 sample.
assay at 10^{-5} .

2/15/50

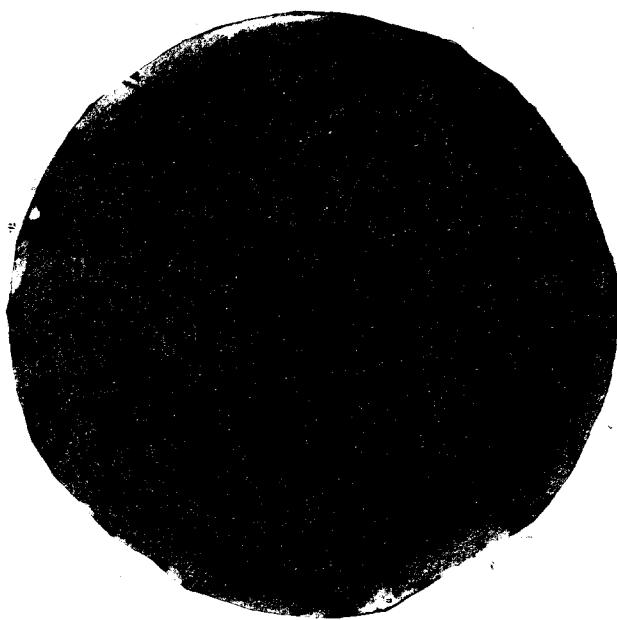
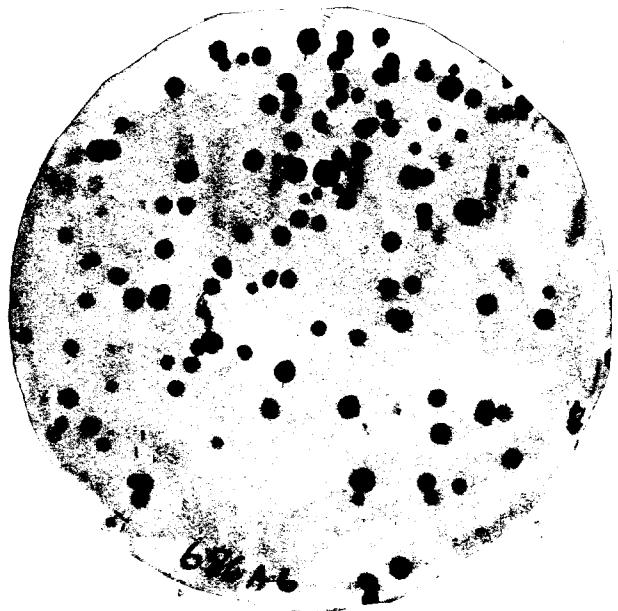
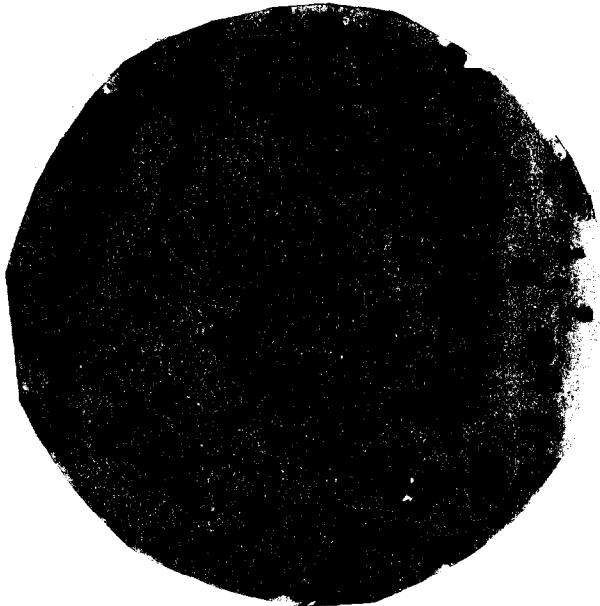
Same suspension

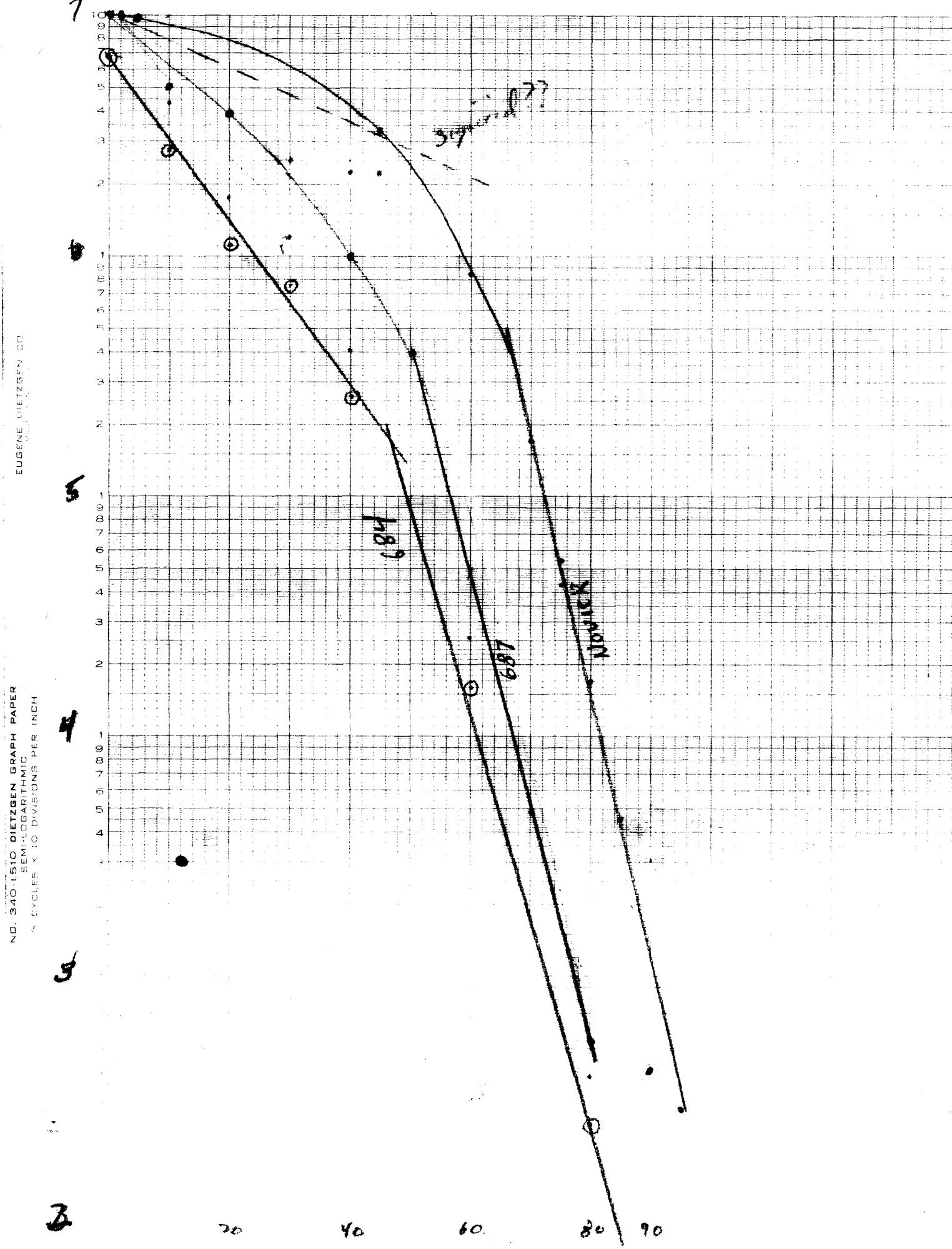
2/14/50.

- A. 1 ml H226 + 9 ml 0% doca in D(-). 11⁴⁵ AM. - 4²⁵ PM.
pH 6.7 galvanometer
- B. 1 ml H226 + 2 ml 0.5% hydroquinone + 7 ml D(-). 4¹⁵ PM. - 5P.M.
= 0.1% HQ
- C. Diluted ^{new} H226 stock 1:100 for irradiation [dil. as original stock]
Assay.
- D. into 10 ml ^{H2O}; 200r/ml streptomyces 4⁵⁵ - 6²⁰

		LacU 57	Lac- 80	Σ 137	Σ Count 1.4×10^8	diff ps
A)	6.					
B)	- 2 : sterile!	72	15	87		
C)	Assay. 7	72	15	87	8.7×10^8	
D)	(strep.) 5	230	30	260	2.6×10^7	

∴ doca again shows only slight killing despite prolonged exposure (almost 5 hours). - slight hydrolization noted. Should be studied for balanced lethals.





uv killing curve: H226

687

2/14/50.

Fresh (24; aer. D(4cc)) stocks of H226. Dilute 10^{-2} to give estimated 10^7 /ml. Irradiate in open dishes, 50cm uv, at 684.

uv-secs.	dil.	LacV	Lac-	Count →	%	v
A 0	5	173	22 { 22 { 14 { 14 { 195	1.9×10^7	1×10^7	89
B 2	5	171; 167; 143	41; 41; 29	197	2.0×10^7	1×10^7
C 5	5	160	82	193	1.9×10^7	1×10^7
D 10	5	<u>91</u>	<u>102, 80</u>	<u>158, 76</u>	2.1×10^7	8×10^7
E 20	4	Too heavy	$\frac{270}{110} = \frac{117}{510}$	780	7.8×10^6	3.9×10^6
F 30	4		$\frac{123}{385} = \frac{385}{123}$	508	5.1×10^6	24
G 40	3	>200	Too heavy. ca 500	? 1500?	2×10^6	ca 30
H 50	3	>200	184	613	8×10^5	4×10^5
I 60	2	>200	Too heavy.			
J 70	2	70	6; 7	98, 79	9.6×10^3	8.48×10^3
K 80	1		12; 8	88	1.1×10^3	9
L 90	1	60	16	128, 70	5.4×10^2	20
			16	99		
			63	79	7.9×10^2	6.0×10^2

119	74	193
78	85	183
104	61	165
121	95	216
109	77	206

551	412	963
110	82	193

C
Sputter.
1
2
3
4
5

D
2 plates
(

served at 24h.

Algescts plated on EMS Lac

A	+	-
	189	0
B	194	= 0
	178	2?
C	120	6
	100	10
D	106	12

Hearn +

Hearn from 687
173

160

110

91

Picks and streaks out apparent - on EMB Mal.

	EMB Lac
1	-
2	-
3	-
4	-
5	+
6	-
7	-
8	-
9	-
10	-
11	-
12	-
13	-
14	-
15	-
16	-
17	-
18	-
19	-
20	-

EMB Mal

v	*
v	*
v	*
-	*
+	*
+	*
v	*
v	*
v?	*
v	*
v	*
+	*
+	*
+	*
v?	*
+	*
v?	*
+	*
+	*
-	*

Xyl

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UV Killing - K-12

688

2-16-50.

Grown in Dlac 24h. aer. store in refr ca 2-3 days.

Dilute 10^{-2} in saline for mediation.

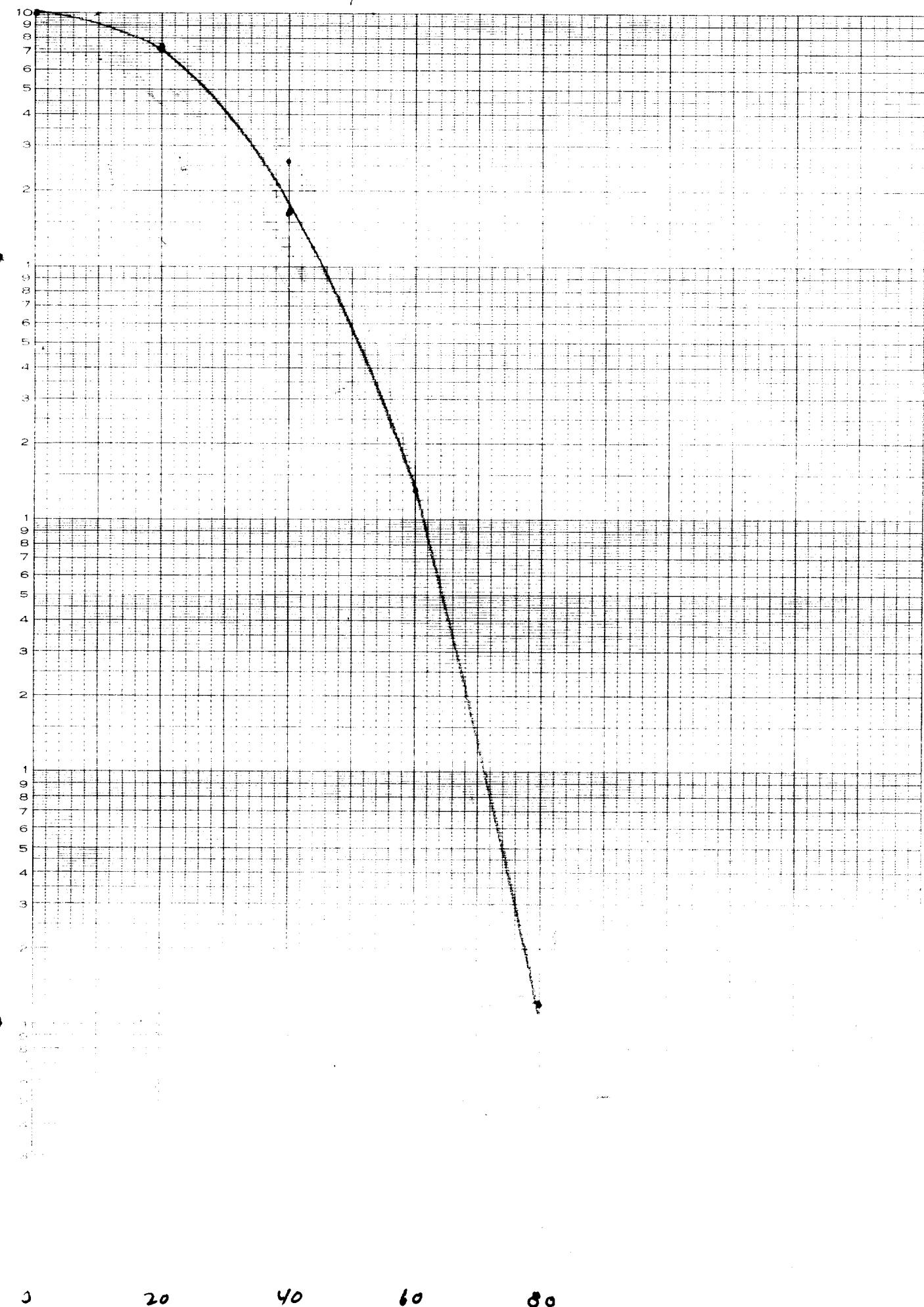
UV sec	Dil	Count		$\div 6.1$
0	5	608	6.1×10^7	1×10^7
20	5	462	4.6×10^7	7.5×10^6
40	4	1600	1.6×10^7	2.6×10^6
60	3	823	8.2×10^5	1.3×10^5
80	1	730	7.3×10^3	1.2×10^3

K-12 UV

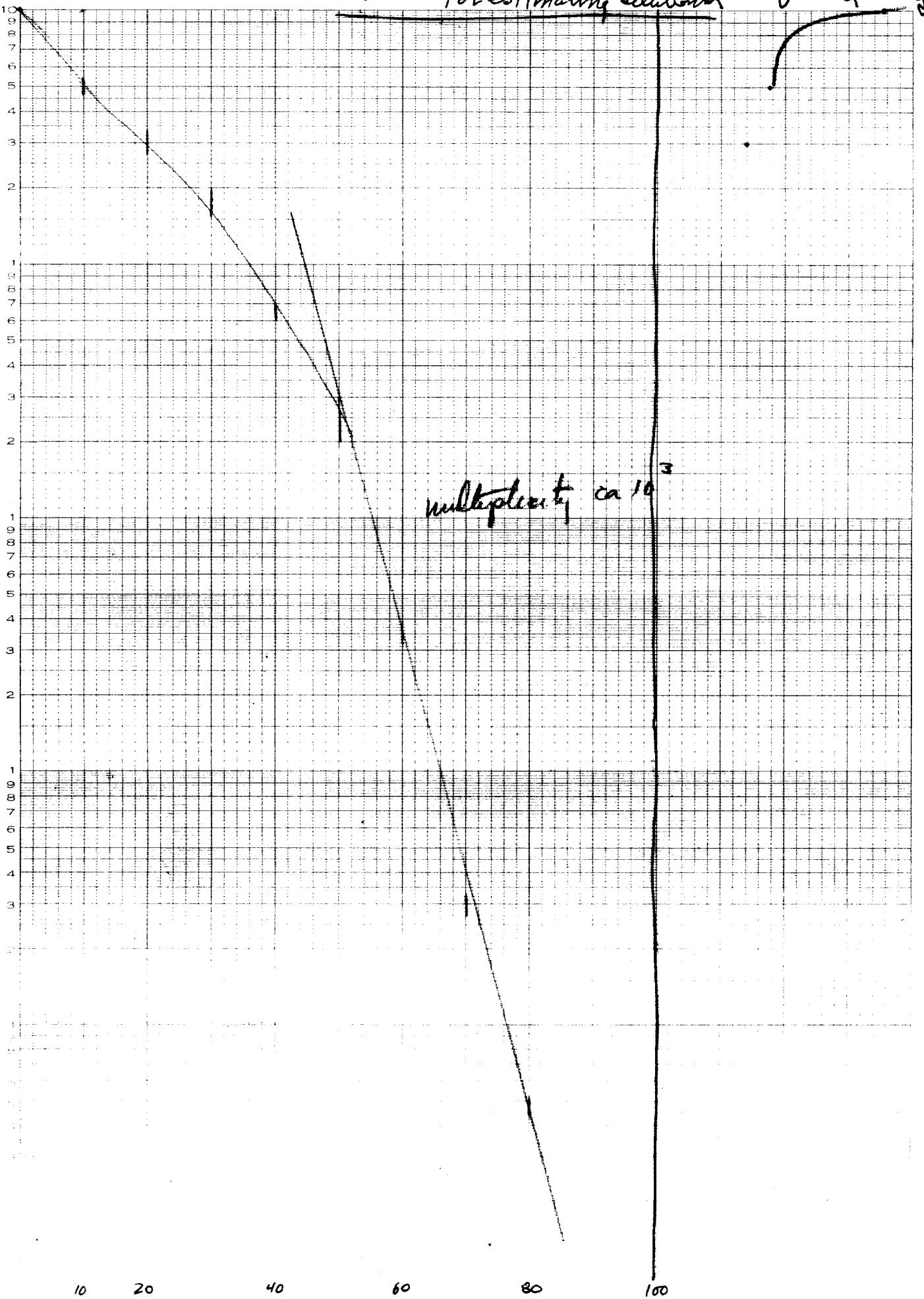
638

EUGENE DIETZGEN CO.
SIXTY-FIVE YEARS OF SERVICE

NO. 340-U510 DIETZGEN GRAPH PAPER
ONE MILOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH



Rough standard - H226
For estimating teletron



Treat H226 with Acetic anhydride

689

2/19/50

Add 0.1ml Ac₂O to 10ml H₂O. Add 1.1ml sterile 10% CaCO₃ suspension. Add 1ml H226 and shake at room temperature.

After 10 m., plate out on EMB lac (original thouroughly assayed.)

[Assume $\approx 3 \times 10^8$ ca 90% surv.]

All plates sterile. See 692 for effect of 0.1% Ac₂O.

2/20/50.

	(2/19/50)			
B	1 ml H226	10 ml H ₂ O	<u>.1 ml</u>	1% Ac ₂ O in 10% alc
C	"	"	.5 ml	"
	10 mins 37°.			

B6	4900 239	495 - 203	/	442.	Count: 4.4×10^8 Survival = $\frac{4.4 \times 10^8}{\times 10^9}$ $\approx 40\%$	(see 694 assay)
A22 ✓						
C2	0	3			Hold for delayed survival	
22	5.6 sterile					
C2	14	106		Hold further		

K12 vs. H226 uv sensitivity

H226 uv; chemicals

2/18/50.

Mix H226 + K12 [2/14], dilute to 4×10^{-7} .

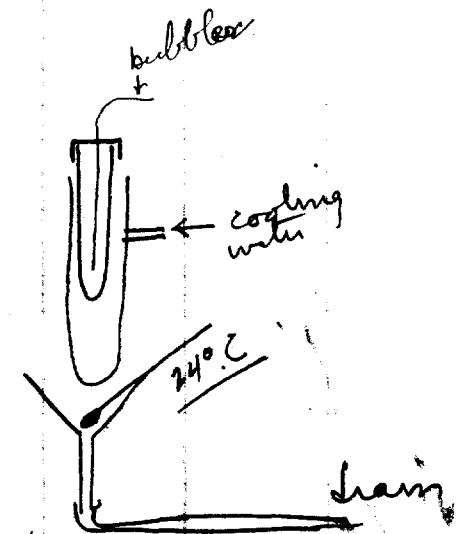
Take 1 ml and spread on EMB Lac

- A) Control
B) UV 20 secs.

C H226 dil 10^{-2} for irradiation.
 10^{-5} assay.

15 ml v.d.

D UV 20×10^{-5}
E UV 80 secs. 10^{-1}
F UV 80 secs. + light. (focus Spencer projector microscope lamp on suspension, in cooling jacket, 12 cm. from lamp aperture).
 $4 \times 10^{-5} \rightarrow 6 \times 10^{-5}$ mlns.



Formaldehyde

1 ml susp. + 8.5 ml H₂O + .5 ml 1% CH₂O (\rightarrow .05% final)
 $5^{\text{min}} - 5^{\text{hr}}$ (= dil. 1) 20 mins. \rightarrow ca 10% surv.
 mortally injured

H - 600 strike 60 mins.

Note: after 40 hours, H3 had ca 10^3 colonies (\therefore this agent may give a delayed recovery).

C. Fruit count was done at ca 24 hours. "loc -" was marked for review at ca $\frac{40}{4}$ hours (10A20) 3 require revisions from loc- to locv.

This alters the means to

$$\frac{478/3}{20/3} = \frac{159}{7} \quad \text{locv : } \frac{\text{locv}}{\text{loc-}} = \frac{\text{ca } 95\%}{5\%}$$

D. lac

	V (+)	-	Σ
1	63	106	
2	54	98	
3	64	87	
	<u>181</u>	<u>291</u>	
3/	60	97	157

Comparative later:

Killing: 47% survival
39% locv.

Compare with D - Mal: 68/166 Malv! Not greatly different.

E. lac
 80 seeds
 10^{-1}

	V (+)	-
1	15	72
2	8	72
3	16	75
4	17	106
5	19	90
	<u>75</u>	<u>415</u>
7	1	18
	0	13

$$\bar{m} = 15 : 83$$

killing

$$5.9 \times 10^{-5} \text{ survivors}$$

18% locv.

= $UV 80s$. lac
+ light. 10^{-4} :
 10^{-5} :

29	54
3	9

$$\frac{\text{Survival}}{80(L)} = \frac{8.3}{166} = .05 \quad 35\% \text{ v}$$

$$= 45(D) \quad f. 387$$

agreement!

Formaldehyde

105% 20 mins.

6

tac +	Lac -
22	45

/ 67

Assay is 6.7×10^7
 original was 1.6×10^9
 $\text{Survival} = 4.2 \times 10^{-2} \approx 5\%$
 ca 33% Lac V.

2/20 Repeat a, b expt.

Mix .05 ml M226 + .05 ml K-12 grown in parallel 20h. O(Lac) aer.
 in 100 ml. ($= 10^{-2}$ dil.)

A. 10^{-5}	Control
B. 10^{-5}	20 secs uv
C. 10^{-1}	8000 sec uv.

150, 122	75, 59	8, 2
45, 62	9, 6	21, 23
126, 144,	7, 7	23, 32

Control :	272 : 144	/ 416	K-12 %	65 or	1.9 : 1
uv 20	107 : 59	/ 166	"	65	1.8 : 1
uv 80	269 : 69	/ 338	"	79	3.9 : 1

i.e., ca 2-fold increase in proportion of K-12 over 4 decades
 of killing!

Dear Josh,

Here's another batch - I don't have too much longer for this run.

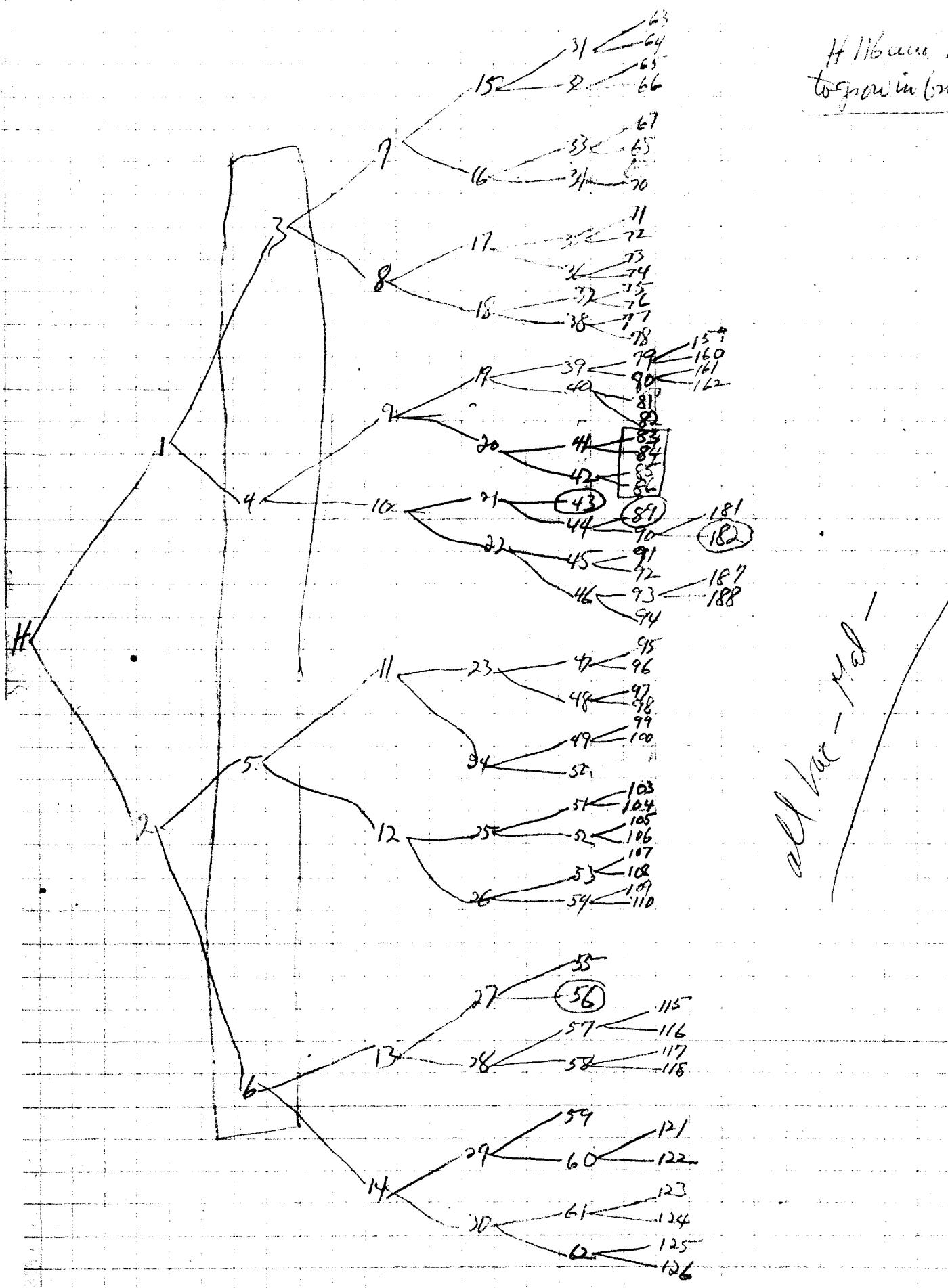
The 8-5-50 pair was interesting - am anxious to hear the details.

Also anxious to learn your reaction to what I wrote. Please excuse me if I overstepped propriety in my last letter. You're going to not send it but didn't get around to writing another one.

I'm planning another session this Wednesday (holiday for D. Wash.) & another for the weekend. So be looking for a couple of more batches soon.

M.W.H.

H116 and H123 found
to grow in both tubes.

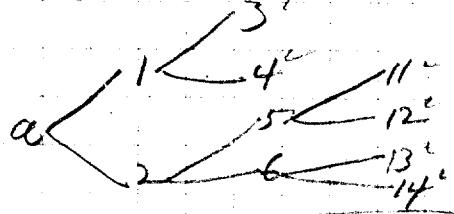


2-18-50

H 226 source.

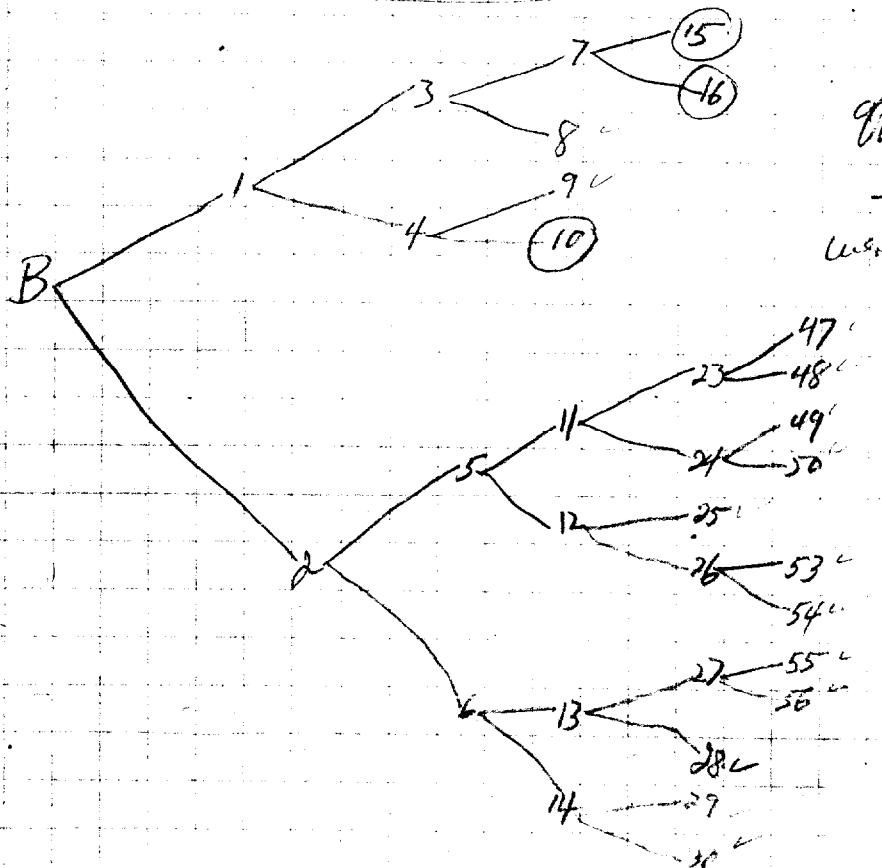
- (○) = didn't grow
- (□) = unknown side relationship

cells tends to become
filamentous - stopped because
of an engagement,
all het

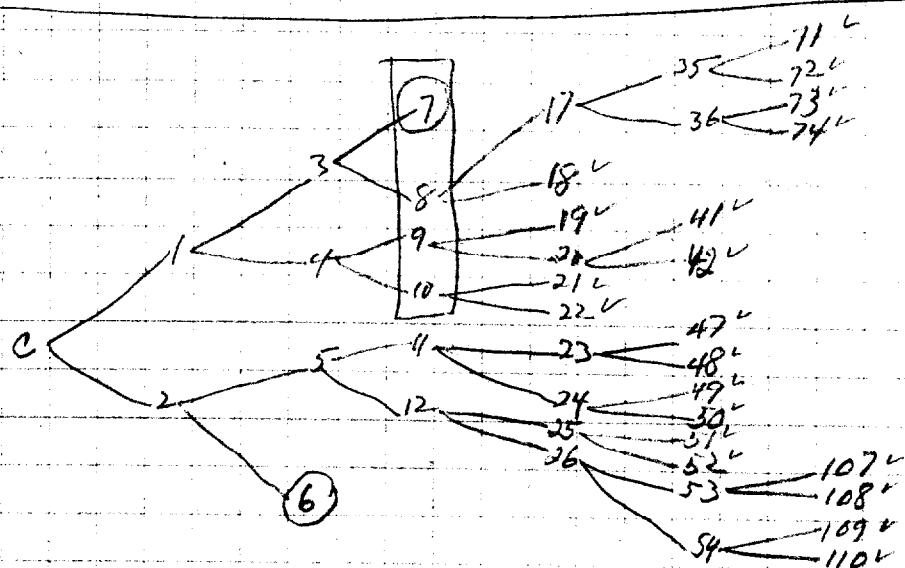


cell 3 was a filament, then split
off small cell at each end

to 15 8 16
were conditionally numbered as indicated

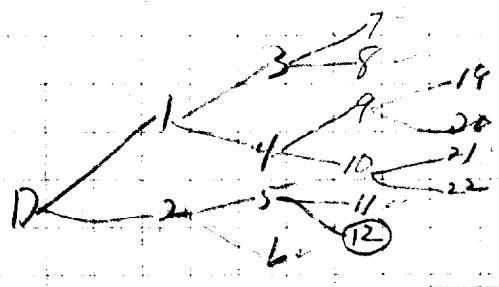


all het

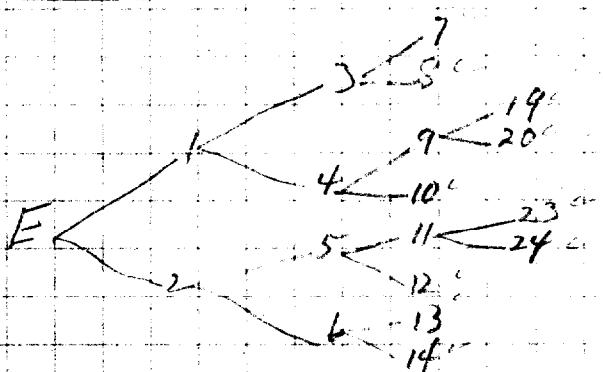


All the microcolonies were
filamentous - round, dense, "dry"
(i.e., hard to pick up in pipette)
like those of clonal of 2-5-52

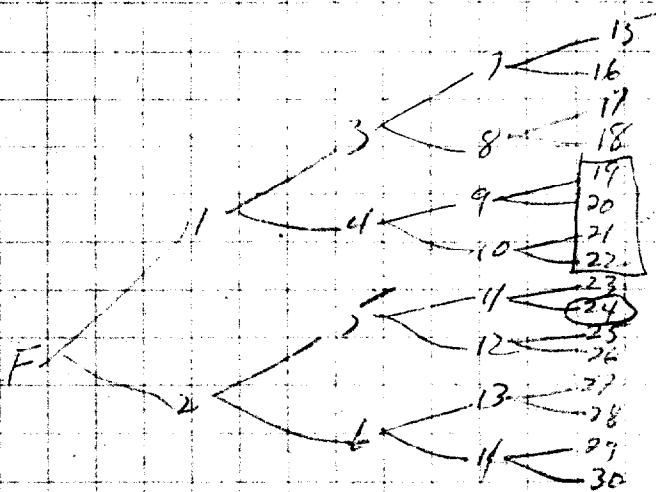
L - M -



all het



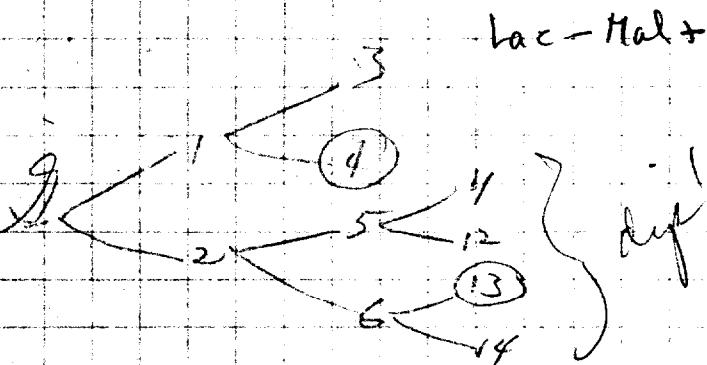
all het



all het

Cells in this clone were very small from the start & would never cell out as a segregant. Went along & used it for the Peltier.

F22 failed to grow in the broth tube due to breaking the tip off the micro-pipette.



all het

lac-Mal+

690d

Platings of UV-H226 on EMS

Febr. 20, 1950.

Platings on "EMS Mal" ~~probably~~ strike medium probably mg.

EMS Lac:

Control

C

154	+	0
136		0
146		0
<hr/>		
436		0

in 145 + : 0 -

D

97	16
126	25
104	31
109	34
<hr/>	
4 / 436	106
<hr/>	
109	27

1/2 : 54 : 13

note absolute increase in Lac- , simulating
"mutations"

E) Many mosaics, making counts difficult ca 20-25% Lac-.

Pick clean Lac- from D and streak out on EMB Mal.

690 rec.

K-12		<u>H226</u>		
Lac+	Lacv	Lac-	V+-	
104	29	11	40	
81	31	15	46	
97	29	19	48	
<u>282</u>	<u>89</u>	<u>45</u>	<u>134</u>	

282	134	416
<u>259</u>	<u>93</u>	<u>352</u>

not sign. different.
31% - 35%

68	6	19	25
109	7	24	31
82	14	23	37
<u>259</u>	<u>27</u>	<u>66</u>	<u>93</u>

C assay
 $10^{-2} \times 10^{-5}$

	Lacv	Lac-	
	164	9	7
	161	7	6
	160	7	6
m	<u>475</u>	<u>25</u>	<u>20</u>
	158	8	7

D av 20

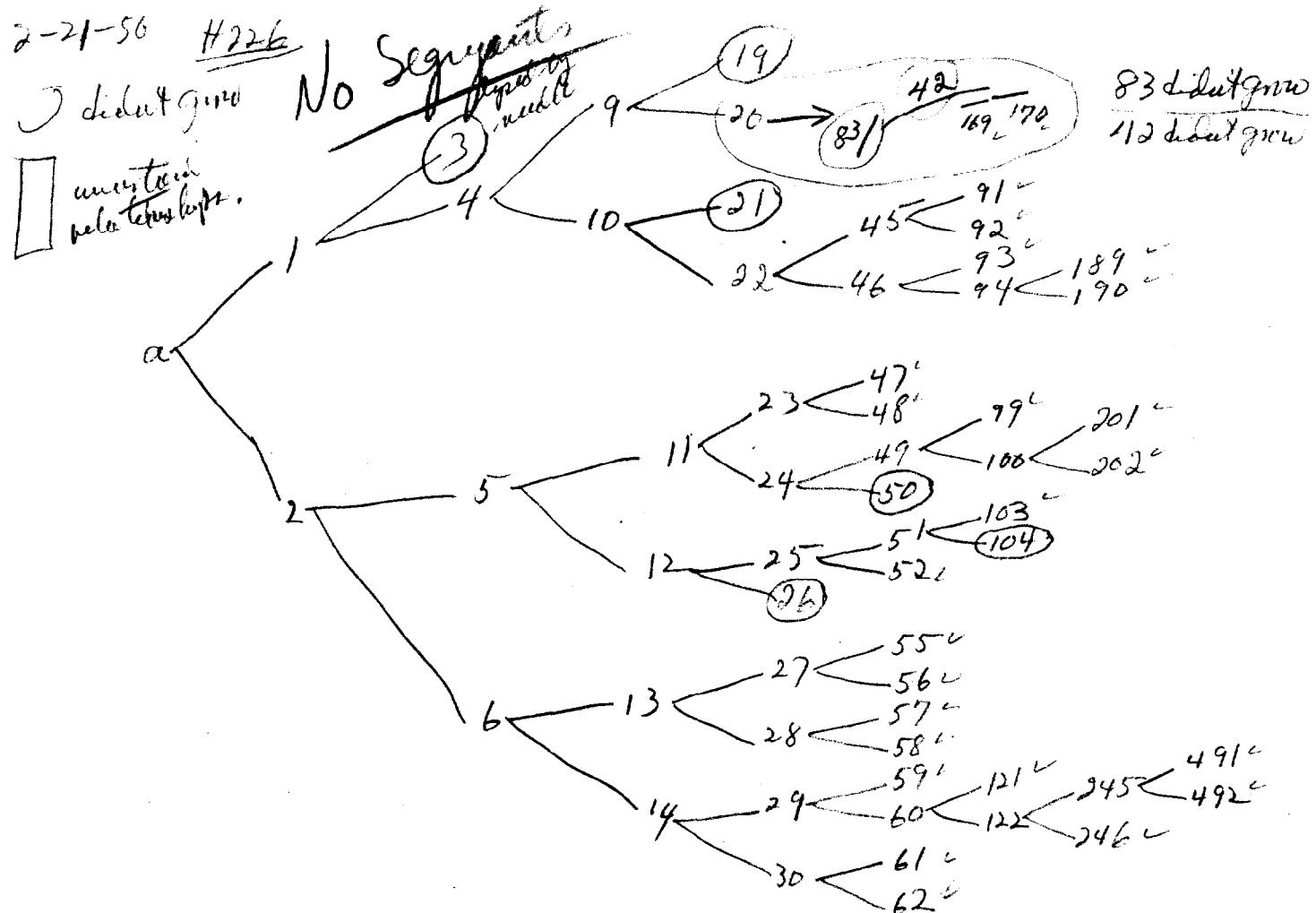
defining counting EMB Lac
many Lacv not yet defined!
+ noted - stuck out as possible balanced lethal type
→ gave Lacv and Lac- only.

EMB/Mal	+	v	-	/	40	/	166
---------	---	---	---	---	----	---	-----

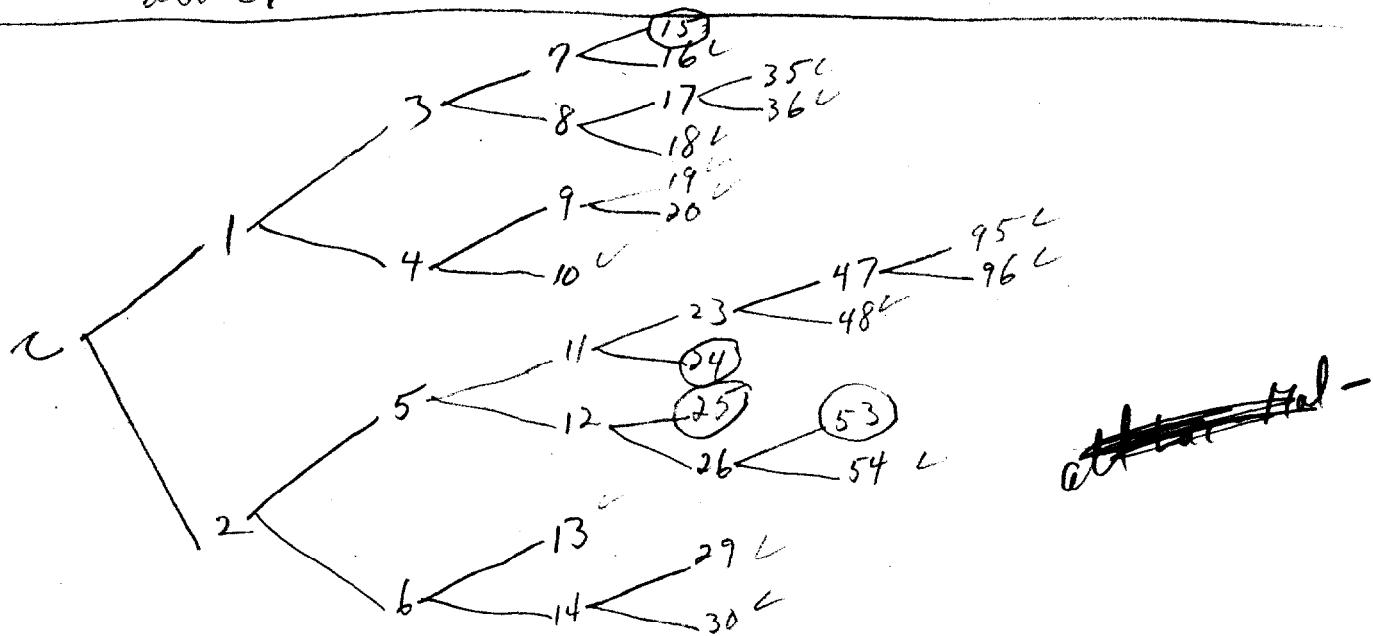
defining

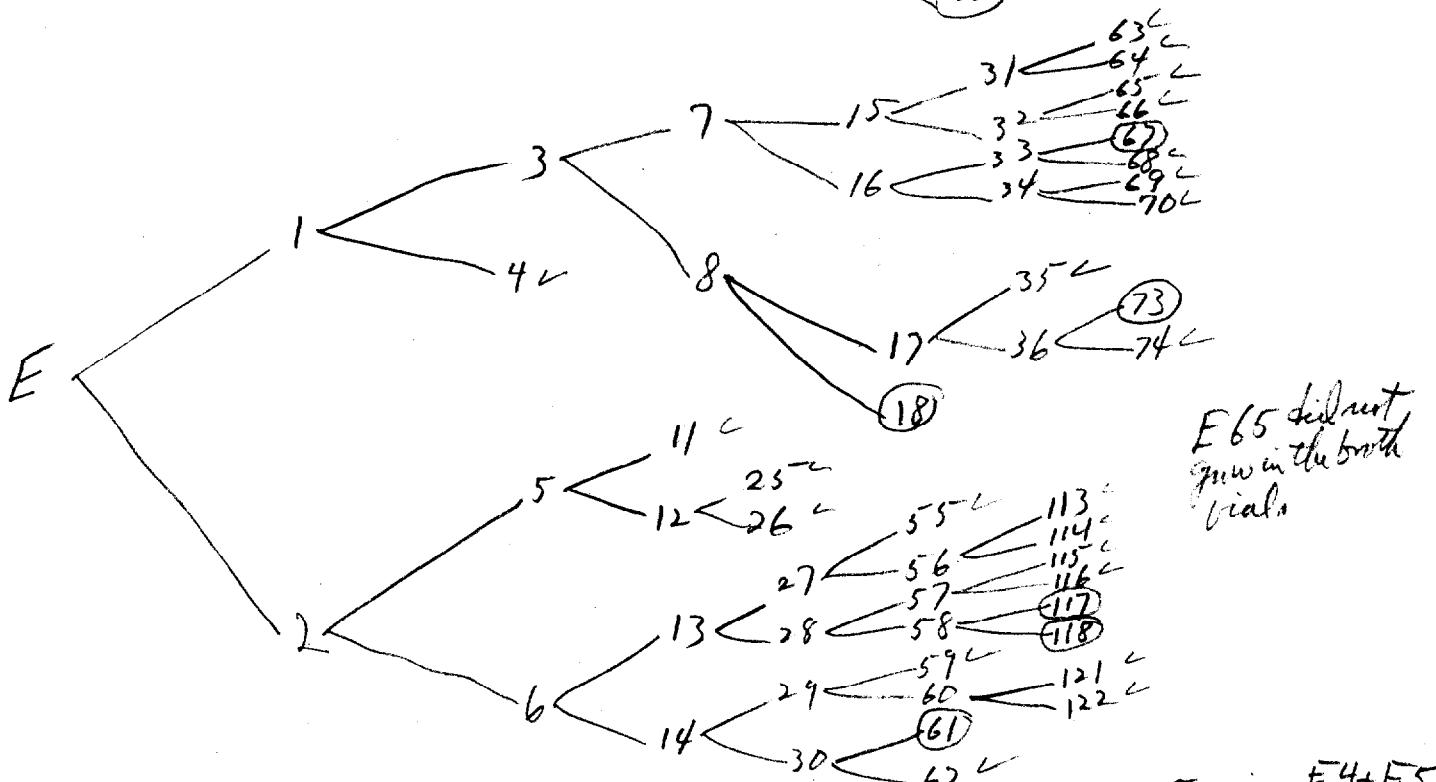
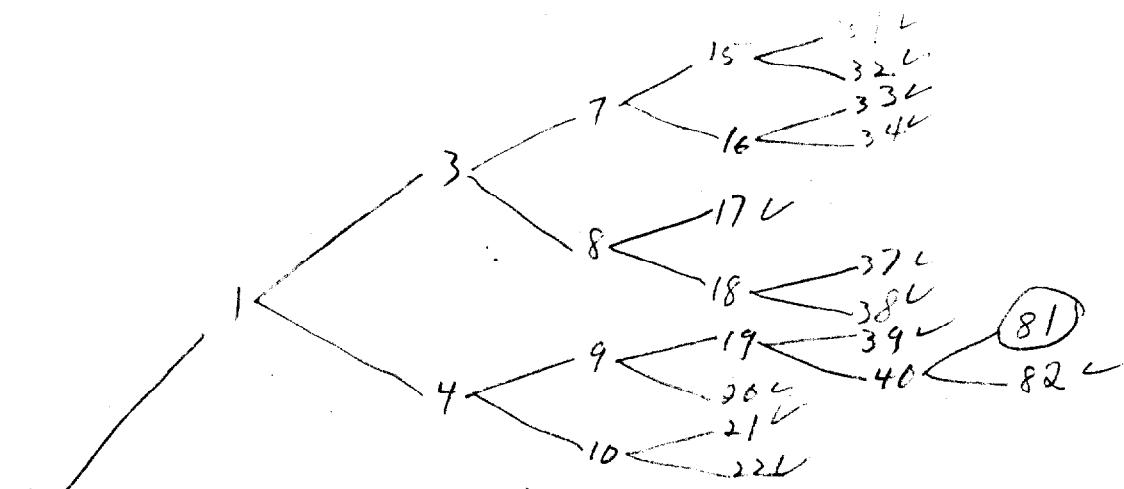
F ca 10^3 + E ca 10%

G



a 20 was a long filament, split off a cell at each end
 one of which divided. Quite arbitrarily numbered them as indicated
 above.





I made an error in my records & had F2 dividing to give F4 + F5
Luckily the actual F4 was not subdivided so I could correctly number the vials with no possible doubt.

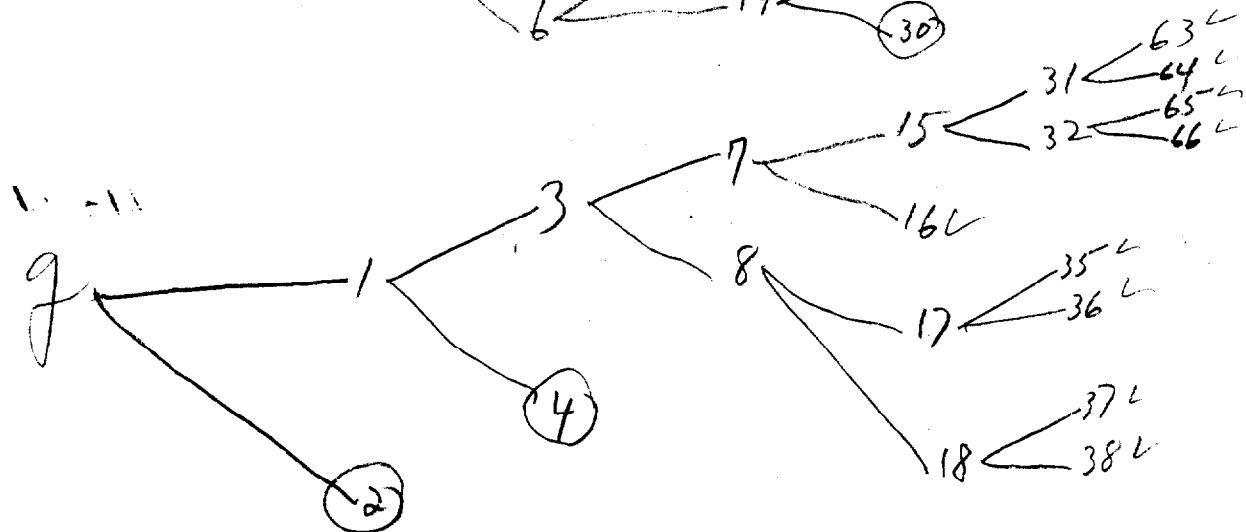
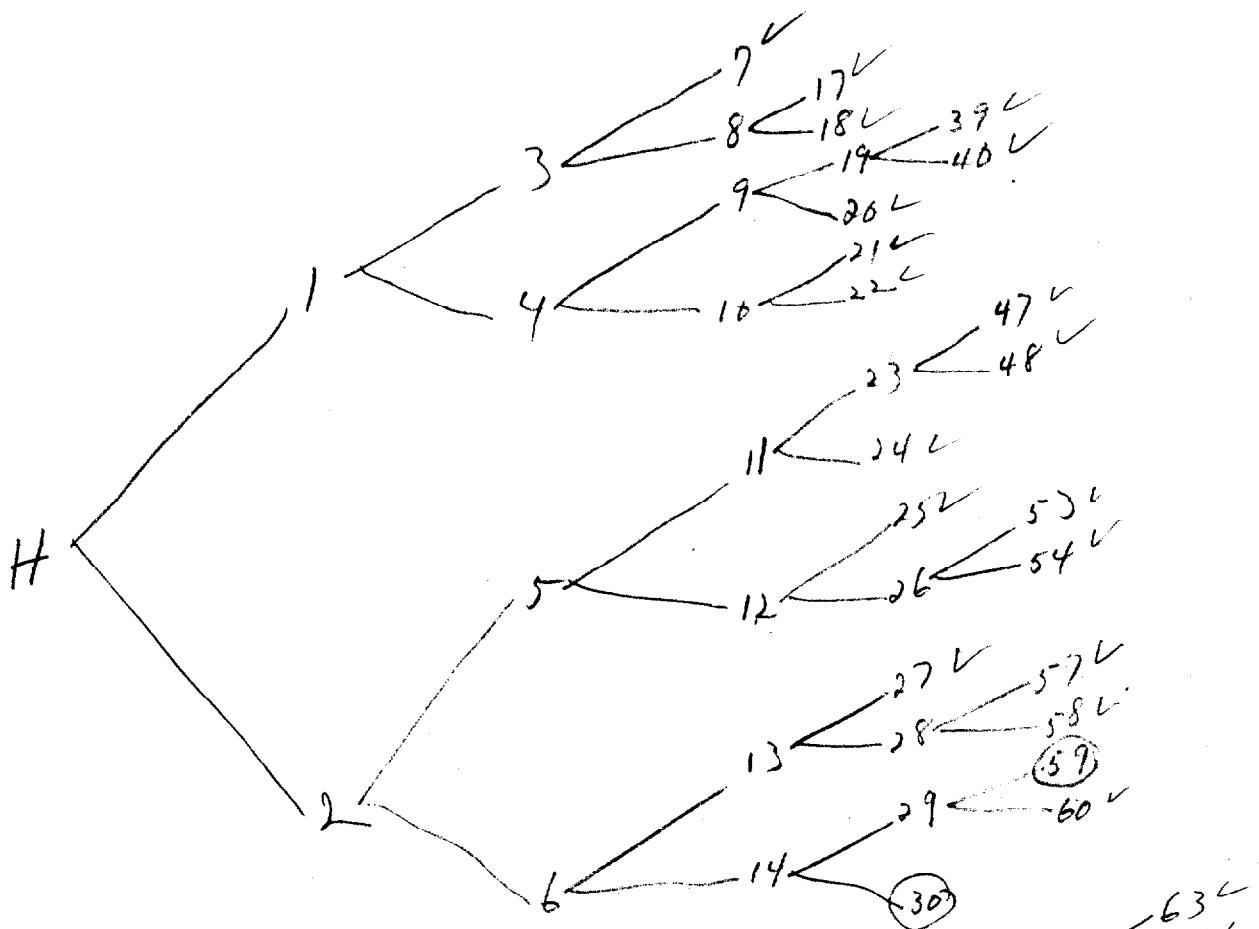
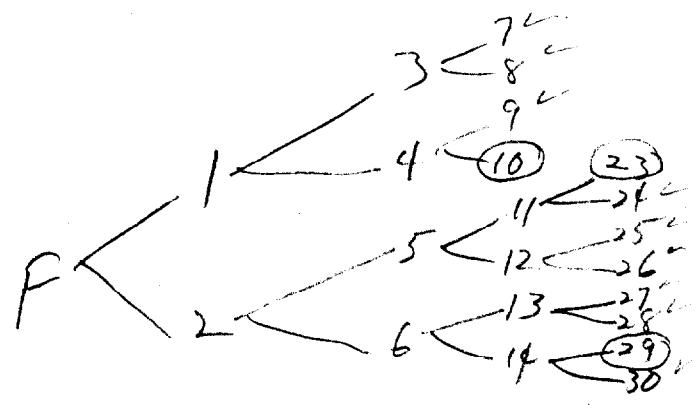
Josh,

I'm still planning another batch for Friday - I don't have
too many hopes for this batch either.

This culture tends to form filaments & then split off
cells on the ends. I'm trying to keep records of all filamentous
cells & sometimes rather arbitrarily number progeny of
filamentous cells indicating how they were numbered.

This is one of the hardest cultures to work with - has a
somewhat longer lag period and few cells from young
EM5 cultures actually grow.

MW



Segregations from H-226

691

2/18/50

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single colonies of H226 nE4B1oc . 4/pl.

Single - colony chosen from each, and tested on
the sugars indicated. Results indicate strongly
the preponderance of λ + segregants (λ c₄ - ??)

Formaldehyde - prolonged exposure.

692

H₂O₂

2/19/50.

A) Formaldehyde
 $\frac{80}{80} \rightarrow 8^{37}$ as 690 G. Use suspension of H226 2/19. Hold!
H₂O₂

B) Expose a 10^{-2} dilution of H226 to decimal stage conc. of H₂O₂ beginning with 30% 1:10. 10ml H₂O + .5ml O(-) + .1ml soap.
 After 15 m. take .1ml samples and spread on EMB lac.

C) H₂O₂
 H226 1ml + 1ml CaCO₃ 10% + 0.1ml 10% Ac₂O/EtOH.
 At 10^{-4} , 198 lac - 0 lac or +! $2 \times 10^6 = 10^{-3}$ survival.

D) assay
 $10^{-3} \rightarrow 1000$ lac - 10^{-2} 7 colonies only!

10^{-7} : lac+ 146 lac- 12 / 156

B	% H ₂ O ₂	lac+	lac-	Notes
1	3	Stable		
2	0.3	Stable		
3	0.03	Stable near control; crowded at periphery. (lac - around center?)		
4	0.003	Crowded!		
5	0.0003	Crowded!		

Higher than .03% bacteriostatic on plate. [Should use catalase.]

A	lac+	lac-	Survival = $\frac{5.4 \times 10^7}{1.6 \times 10^9} = 3 \times 10^{-2}$
P2 3 complete A22	6		lac only 6% liquid
	3	51	
	27	53	
5	13	-	
A22	>>13		

Suggestions! Do prolonged doses of chemicals differ from UV in permitting a much lower proportion of diploid survivors, conforming to nuclear elimination theory of killing.

Segregation of H226

693

Febr. 20, 1950.

B. 130 Dose. H226 1:10 in Pernasay; aerate to 7¹⁰ (6 hours). Plate out on EMB Mal. → But mostly still lac+! (ca 80%).

A. Streak out single lac+ colonies of H226 (690C) on EMB Mal.

Pick single Mal+, Mal- colonies to nudge and spot on EMB lac, Mal, Xyl, MHP.

2/21 (A) 57 Mal- : all lac- MHP-Xyl-

62 Mal+ : 32 lac+(v), MHP+ Xyl+ ... 32 lac+ MHP+ Xyl+

3 lac- MHP-Xyl-

5 lac- MHP-Xyl+

All above tested: V₁^R.

[do H226 V₁^R/V₁^S?]

But segregants are preponderantly parental combinations.

[Tests of phage resistance; mutators are needed.]

(B) Mal- picked (dry M.O.)

Lac	Mal	Arg	MHP	L	M	X
-	-	-	-	11 (2)	-	+
3	±	±	-	12	-	+
4	±	±	-	13 ±	±	±
5	±	±	-	14 ±	±	±
6	+	+	-	15 ✓	-	-
7	⊕	⊕	-	16 ✓	-	-
8	-	-	-	17 ±	±	+
9	-	-	-	18 ✓	-	-
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March 4, 1950.

In older cultures of H226, a number of "partial segregants" have been isolated. See also 687: 9 Mal^v Lac- were picked up following ~~H226~~ irradiations. Later, the same was found in ~~H226~~ control cultures.

693 (2/22) 4 Lac^v Mal- and 1 possible Lac-Mal^v isolated.
from EMB.

3/1

c. See 700. Plating of H226 after growth on EMB/Mal.

10 Mal^v (?) picked. Strains on EMB/Mal, Lac.
all were Lac^v Mal^v.

D. See 700 + 1. on EMS/Lac, Mal. <1% - . Repick and purify as same medium for subsequent testing.

Mal- : 8 ; 3 Lac^v. #2, 3, 8. = 200 H, 12, 13

699:17, 18, 19

Lac- : 14 2 Mal-; 12 Mal+ No Mal^v.

Segregation of H226
Partial segregations?

693a

2/22/50.

See 693(B).

1-4 from Lac (693B: 11, 23, 26, 37); 5-9 from Mal (693B: 6, 7, 21, 25, 39).

Streak out on EMBS Mal, Lac; ETYS Mal, Lac.

Lac EMBS	Mal EMBS	ETYS ^{Lac or Mal}
1 Lac _v ; Lac-	Pure Mal -	+ -
2 Lac _v ; Lac-	Pure Mal -	+ -
3 Lac _v ; Lac-	Pure Mal -	+ -
4 Lac _v ; Lac-	Pure Mal -	+ -
5 Lac-	Mal+; Mal- v?	ng n.g.
6 Lac-; Lac+(+?)	"	+ +
7 Lac-	"	ng n.g.
8 Lac-	Mal+ - v?	ng n.g.
9 Lac-	Mal- v?	+ n.g.

These isolates bear no doubt as to the occurrence of Lac_v; Mal- types. How do they arise? They would represent a persistence of the 2 stage induction noticed by Zelle. A Mal x Lac- has also been picked up.

H226 partial segregation.

6936

2/24

Grow H226 1:100 to saturation in Ringersay (aerated)

2/27 Plate at 10^{-5} m EMS, EMBS 1% Mal and lac.

EMS: ca 100 prototrophs +, and -

EMBS: Turbid!

2/28 Plate at 10^{-7} (+) m EMBS.

Lac EMBS: ca 1% deploid; remainder are lac -

Mal EMBS: mostly Mal+; a few Mal-, Mal_v.

A. Pick - from Mal EMBS. Brush on Mal EMS, streak on Lac, 1% EMBS

B. - Lac " Mal EMBS " "

C. Pick lac_v from Lac EMBS. Streak on Mal EMBS; same suspensions.

D. Pick Mal_v from Mal EMBS. ~~Streak~~ Brush on Lac EMBS. ~~2~~ ^{only} 2 scorable at this time!
both lac_v.

See p. 698

~~200~~ + 3/1

A. 11 "Mal-"
from EMS.

	Lac	EMB	Mal
1	v	-	+
2	+	-	+
3	v	-	+
4	v	-	+
5	v	-	+
6	-	-	+
7	v	v	-
8	v	-	+
9	v	-	+
10	v	-	+
11	v	-	+

B. 38 "Lac-"
from EMS

1	-	+
2	-	+
3	-	+
4	-	+
5	-	+
6	-	+
7	-	✓
8	-	+
9	-	+
10	-	+
11	-	+
12	-	+
13	-	+
14	-	+
15	-	+
16	-	+
17	-	+
18	-	+
19	-	+
20	-	+
21	-	+
22	-	+
23	-	+
24	-	+
25	-	+
26	-	+
27	-	+
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32	-	+
33	-	+
34	-	+

35	-	+
36	-	+
37	-	+
38	-	+

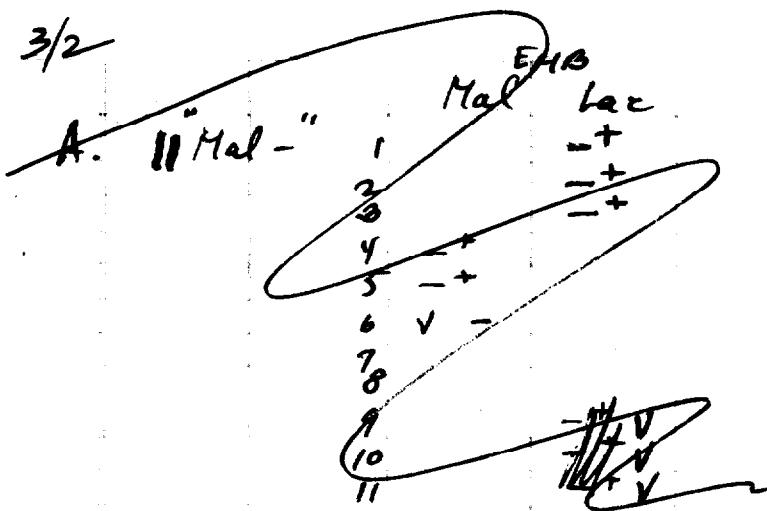
The frequent occurrence of (Mal-Lac v) amalgam Mal- prototrophs is indubitable. The residue of Mal+ popillae is not explained.

In some cases here, a single colony probably contains Mal- and Malv, lacy.

A single essentially Lac- Malv culture has been recovered here.

Apparently, most prototrophic segregants are Lac- Malv.

3/2



C. Lac to Mal.

1	✓	11	✓	21	✓	31	✓	41	✓
2	✓✓	12	✓	22	✓	32	✓	42	✓
3	✓✓	13	✓	23	✓	33	✓	43	✓
4	✓✓	14	✓	24	✓	34	✓✓	44	✓✓
5	✓✓	15	✓	25	✓	35	✓✓	45	✓✓
6	✓✓	16	✓	26	✓	36	✓	46	✓✓
7	✓✓	17	✓	27	✓	37	+	47	✓
8	✓✓	18	✓	28	✓	38	+-	48	✓
9	✓	19	✓	29	✓	39	+-✓	49	✓
10	✓	20	✓	30	✓	40	✓	50	✓

Not well streaked out. The cyst.

No Lac & Mal- found should be repeated.

Photorecovery of uv effects

694

2/20/50.

See 690 for set-up. Irradiate 2/19 H226 10^{-3} 5 seconds.

- A) No uv
- B) uv 5 sec.
- C) uv 5 sec +

light 110 ~~—~~.

(temperature not
well controlled)

C: no survivors.

Count A + B at 36 h.

	Lacv	Lac-
①	63	16
	91	30
	74	18
	<hr/>	<hr/>
	228	64

	Lacv	Lac-
②	65	31
	79	37
	30	14
	<hr/>	<hr/>
	174	82

increased pop of O types.

Relatively little effect at 5 secs. (use 10 for
following expts.)

2/22/50:

A:

	Lacv	Lac-
	114	9
	134	2
	124	<hr/>
	<hr/>	<hr/>
	372	17
	<hr/>	<hr/>
	124	6
	<hr/>	<hr/>
	74	37
	<hr/>	<hr/>
	61	57
	<hr/>	<hr/>
	44	33
	<hr/>	<hr/>
	62	41
	<hr/>	<hr/>
	241	168
	<hr/>	<hr/>
	60	42
	<hr/>	<hr/>
		102

B

	Lacv	Lac-
	69	10
	74	16
	91	25
	<hr/>	<hr/>
	234	51
	<hr/>	<hr/>
	78	17
	<hr/>	<hr/>
		95

Tests for balanced lethals

2/21/60 ff.

Pick lac⁺ colonies from a variety of treatments to examine for balanced lethals. Streak out on EMBO lac⁺, and examine leucine + for stability. If any are more or less unstable, re streak. Cephazyme inhibits lac⁺ (so this frequency is exaggerated)

690D: was tach.

690D: 28 colonies. 10 Rechecks

E. ¹⁷~~20~~ cols. 10 Rechecks. 1v = 695-3

F. 12 cols. 4(1a,b) Rechecks. 1v = 695-2

G. 4 cols. 0 checks.

692A 12 cols. 1 pure lac⁺⁺ transferred to slant. as 695-92A1. others tach.

689B

* Repick from first streaking if any colony not obviously sectoring is seen.

Re-tests for possible partial segregants

696

March 6-7, 1950.

A.	1	= A 169	Lac	Mal	Mfe	Xyl
=2/21/50	2	A 189		++	+ -	✓
series.	3	C 30		✓	+ -	✓
	4	E 116		✓	+ -	✓
	5	E 121		++	-	✓

2/24	7	A 15	++	✓	✓	
	8	B 21	✓	✓	✓	++-
B	9	B 36	++	++	++	
	10	C 25	✓	✓	✓	
	11	D 12	✓	✓	✓	
	12	D 13	✓	+(-)	++	+-
	13	H 22	✓	++	++	++
	14	I 26	+	+	-	++
	15	J 16	+	-	+	-
	16	K 16	✓	++	++	
	17	K 22	++	✓	++	-
	18	L 13	++	-	✓	-
	19	F 10	++	++	++	
	20	F 12	++	++	-	++
	21	F 15	++	++	++	
	22	F 16	++	++	++	
	23	F 19	++	++	-	++
	24	F 24	++	++	-	++
	25	F 27	++	-	✓	++
	26	F 28	++	✓	++	-
	27	F 29	++	-	++	-
	28	F 30	++	++	++	
	29	F 42	+	-	++	-
	30	F 47	+	-	++	-
	31	F 48	++	++	++	

F series (19-31) is peculiar in Lac+ Mal+ (very few - segregants). Isolate and compare isolated diploid with H226.

of the others : 2/24 : B 36, D 13; H 22 and K 16 warrant detailed attention. In 2/21, A 189 and E 121 should be isolated.

EMB Mal, Lac

EMB Lac

696

Zelle 218.

~~Hest~~

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all -
bar - Hal -

Most of these pedigrees are uninteresting.
However, keep B28, 55, 56; Series D; E 7, 8, 10, 20;
~~E~~ Series G;

so key 680: A15; A33

D:15-22; 53; 27,29

E 215-222; 108, 49, 52, 75, 76

6: entries per line

Zelle - single cell pedigrees

696

Zelle 2/18

	Lac	Mal
7	+	+
8	+	+
10	+	+
12	+	+
13	+	+
14	+	+
19	+	+
20	+	+
21	+	+
22	+	+
23	+	+
24	+	+
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Σpure? Der Rekurs: $\begin{matrix} --, v \\ --, v \\ ++, v, - \\ ++, - \end{matrix}$

all Lac -

all Mal -

3/1 3.2/24

I 17 bac Mal Xgl M+L

17	+
18	+
19	+
—	+
20	+
21	+
22	+
24	+
25	+
26	+
27	+
28	+
31	+
32	+
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33	+
34	+
47	+
48	+
59	+
60	+
61	+
62	+

L			X	gl
2			±	+
13	+	+	±	+
14	-	+	-	-
23	+	+	±	+
24	+	+	±	±
25	+	+	±	±
			non-confl	
			g	

~~H22 - some 1422, #26, K16
13
1321, 1336, K22, H22, D12, 13
K16,~~

H22

~~Rectangular~~

Restock small sugars:

A15, B36, A21, C25, D12, D13, F-,
H22, I26, J16, K16^{K22}, L13

K5 K18 413, 14.

~~Larv Malu~~
[SIB IS LETHAL.]

Lacy Haly

3/1 3.2/2

Pedigree H226 Zelle

	Lac	Mal	Xyl	M
9	+	+	+	+
13	++	+	+	+
15	++	+	+	+
16	++	+	+	+
18	++	+	+	+
21	++	+	+	+
23	++	+	+	+
24	++	+	+	+
26	++	+	+	+
29	++	+	+	+
30	++	+	+	+
35	++	+	+	+
36	++	+	+	+
46	++	+	+	+
51	++	+	+	+
52	++	+	+	+
15	+	+	+	+
16	+	+	+	+
18	+	+	+	+
19	+	+	+	+
20	+	+	+	+
21	+	+	+	+
22	+	+	+	+
28	-	-	-	+
29	-	-	-	?
30	-	-	-	+
35	-	-	-	+
36	-	-	-	+
47	-	-	-	+
48	-	-	-	+
49	-	-	-	+
50	-	-	-	+
15	+	+	+	+
17	+	+	+	+
18	+	+	+	+
19	+	+	+	+
20	+	+	+	+
22	+	+	+	+
23	+	+	+	+
24	+	+	+	+
25	+	+	+	+
26	+	+	+	+
27	+	+	+	+
28	+	+	+	+
33	+	+	+	+
34	+	+	+	+
59	+	+	+	+
60	+	+	+	+

Relationships uncertain.

Detailed tests on single cell segregants

696'

March 7, 1950

696A.

A189? streak on

F121 S EMBS Mal, Lac. Both are mostly segregated Lac-, Mal+ but show some Lac+ and Mal+ colonies.

696B. LacEMBS MalEMBS

Resistors (Lac+ [EMBS]).

B36	mostly -	++	✓
D13	mostly -	++	✓
H22	"	++	✓
K16	"	++	+ ✓ -

Recovered
from EMBS lac
or EMBS lac

Fusus	"	"	Mal
10	- +	++ -	++ ✓ -
12	- +	++ -	+ ✓ -
15	-	++ -	✓
16	- +	++ -	✓
19	-	- +	✓
24	- +	++ -	✓
27	- +	++ -	+ ✓ -
28	- +	++ -	✓
29	- +	++ -	✓
30	- +	++ -	✓
42	- +	++ -	+ ✓ -
47	- , +	++ -	✓
48	- , +	++ -	+ ✓ -

Resistors lac+ from EMBS lac where available.

Fusus may have a higher proportion of Mal+, but this is doubtful.
Keep F10

Febr. 24, 1950.

H226 (2/24) brought to Chicago (Inst Radiobiology and Biophysics).
15 ml in small crystallizing dish, shaken gently, exposed to unfiltered
X-radiation. 2 ml samples removed at intervals of 1 min., 10 min., 20 min., 30 min.
and 40 min. 1 minute = 942 ro [(81; 76)]

Plate at various dilutions on EMB Lac. Plates carried from Site B
to Site A in cold weather: temperature shock should be avoided.
~~The~~ Residue of aliquots stored in refrigerator in screw vials.

X-ray effects

2/27-28/50.

Material of 4 of C. X-ray experiment No. _____. 40 minute (ca 40,000 μ) H₂S2/27. Plate 10⁻⁵ and 10⁻⁴ on EMS. 10⁻⁵ on EMBS.

EMB/Lac	Lac+	Lac-	Hal:	+	-	v
0	13			2	5	5
8	14			4	0	5
1	8			1	4	4
1	12			1	4	1
	10	47		6	5	1
				14	18	16

EMS Lac hold!

2/28/50.

10⁻⁴ ("x40-g)

Lac	v (+)	-	Hal	+	-	v
	13	115		67	72	55
	17	126		5	11	7
			10 ⁻⁵	55	1	7
				3	1	5
				2	4	4
				3	6	4

Mal is difficult to count, but proportion of Mal +/- colonies is clear.

A)

Pick clear Mal+ colonies and streaks on Lac, Mal EMBS; ~~lac~~ Mal EMS

B)

Possible lac+ were picked in Chicago for streaking out in Madison. In error, different doses were not separated. 7: 10,000 μ . 9: 20,000 34: 30,000 4: 40,000

A:

	Lac	Mal		Lac	Mal
1	-	+-		9	+-
2	+	-		10	+-
3	-	+-		"	v?
4	+	-		12	+-
5	-	+		13	+-
6	-	+-		14	+-
7	-	+		15	+-
8	-	+		16	+-
17	+	+-		17	+-
18	+	+		18	+-

B: 6 cultures were scored as stable lac+ after two restreakings. They do not appear to be typical lac+ Lac+ hybrids. Store on slants.

698

3/3/50

C. Bells 40 lac - (no apparent +) colonies form plating of
40 min (\approx 40,000 r) x-ray H226. Streak on EGB Mal, and
brush on EMS Mal.

4	+
12	+
13	+
14	-
15	+
16	-
17	-
18	-
19	-
20	+

2
30

$$\begin{array}{r} 1111 \\ + 111 \\ \hline 1111 \end{array}$$

丁乙

ca 10% of
loc - are Mal? %

698

 $0 - 10^{-7}$

Lac v	Lac -
108	7
111	8
119	4
107	7

% V
94

445	26	/	471	118	118	<u>94</u>
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$I_{min} \times Ray = 1000 \Omega$.
 10^{-7}

74	29
68	29
80	23
74	30

296	111	/	407	102	102	<u>72</u>
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10 min. 2×10^{-7}

17	67
15	59
17	71
11	57

60	254	/	314	39	39	<u>19</u>
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20 min 10^{-6}

19	112
30	102
27	121
34	140

110	475	/	585	585	↑
75	610	/	146	1,166	14.6
			685	.86	

30 min 2×10^{-5}

9	73
3	31
3	37
3	36

4.9 9.3

698

$$10^{-7} \times \frac{471}{4}$$

$$1.2 \times 10^9$$

survival % v
1.0

$$10^{-7} \times \frac{407}{4}$$

$$1.02 \times 10^9$$

.85

~~$$K \times 10^{-7} \times \frac{314}{4 \times 2}$$~~

$$3.9 \times 10^8$$

.32

$$10^{-6} \cdot \frac{585}{4}$$

$$1.46 \times 10^8$$

.121

$$10^5 \cdot \frac{685}{2}$$

$$3.4 \times 10^7$$

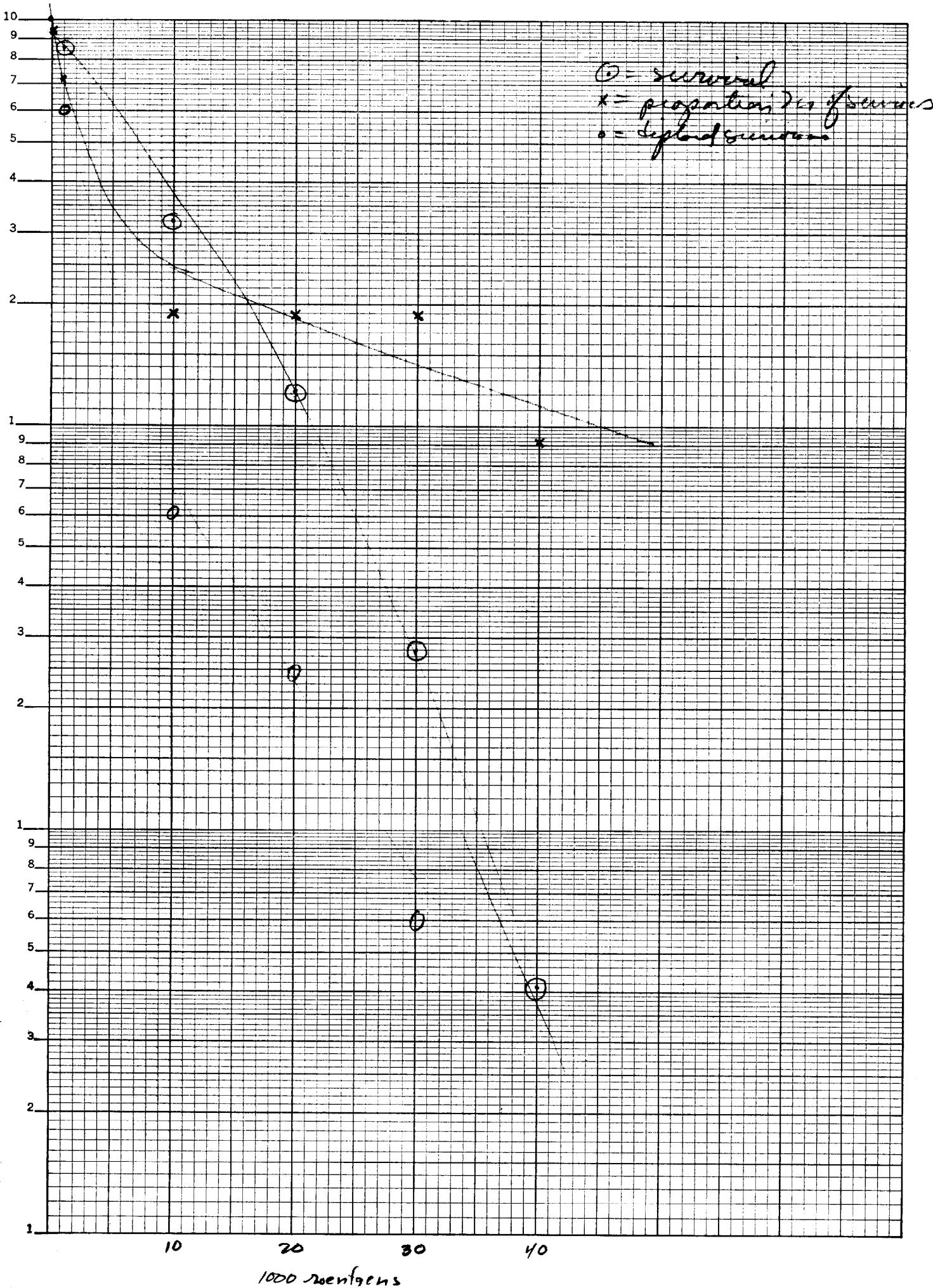
.028

$$10^5 \cdot \frac{195}{4}$$

$$4.9 \times 10^6$$

.0041

698



March 4, 1950.

Group(s) from 693 a 2/22/50. 1-4 contains lac₊ Mal₋. Pick single lac₊ from EMS lac and streak out on Mal; put on plants. = 699: 1-4

6, 5	may also be lac-Mal _v .	<u>699: 5</u>
3/1	693 B. streak on EMS Mal.	<u>699: 6</u>
693 A. 1, 3, 4, 5, 7, 8, 9, 10, 11.	EMS lac	<u>699: 7-15</u>
H227 (See 668 J)	<u>lac_v Mal₋</u> .	<u>699: 16</u>

Re-purify all cultures on EMS lac or Mal.

699:	From	EMS Mal	Reported:			Xyl	Mtl	Mal	Loc
			Mal	lac	Xyl				
1	693a 6	all+	V	V	-	V	-	-	-
2	693B	+,-	V	+	-	-	-	-	-
3	H227	-	-	V	V	V	V	V	V
4	693A 1	-,+ 3	-	V	V	V	V	V	V
5	4	-	-	V	V	V	V	V	V
6	5	-	-	V	V	V	V	V	V
7	7	-	-	V	V	V	V	V	V
8	8	-	-	V	V	V	V	V	V
9	9	-	-	V	V	V	V	V	V
10	10	-	-	V	V	V	V	V	V
11	11	-	-	V	V	V	V	V	V
12	11	-	-	V	V	V	V	V	V
13	693a 1	-	-	V	V	V	V	V	V
14	2	-	-	V	V	V	V	V	V
15	3	-	-	V	V	V	V	V	V
16	4	-	--	V	V	V	V	V	V

P6: Pick 1 colony from EMS and streak out to establish purified stock for reversion tests, etc. Results in pencil
 #1 and 2 require further isolation for Mal_v lac-type
 #5 and 6 require further isolation for Mal- lac_v.

Abundance of lac-Mal_v is seen.

Partial segregants.

March 10, 1950.

(EMS)

#1 Pick several Mal+ colonies to EMBS Mal. of 12, #2, 7, 8, 12.
 Restreak as EMBS Mal as potential Mal_v Lac⁻: confirmed.

#2 Only Mal+ and Mal- found on restreaking.

#5.6 6 Lac⁺ (v?) Mal- ~~OK~~ N.G.

#6 5 =4 Lac_v Mal- OK Lac_v Mal-

Test these cultures:

	bac	Mal	Mtl	Xyl
1	-	v	v	v
3	v	-	v	v
4	v	-	v	v
5	v	-	v	v
7	v	-	v	v
8	v	-	v	v
9	v	-	-	-
10	v	-	v	v
11	v	-	v	v
12	v	-	v	-
13	v	-	v	v
14	v	-	v	v
15	v	-	v	v
16	v	-	v	v
17	v	-	v	v
18	v	-	-	-
19	v	-	v	v
20	-	v	v	v
21	-	v	v	v

Mal+ recessions:

Type 1

3

1. Mal_v Lac_v - pur?
Stable

4

1. Mal+ Lac-

=712A1
A2

P15

699-11 Mal⁺ ^R carry as 699-11R1. Lac_v Mal_v⁻

P17

11b. Lac⁻ Mal⁺. Segregant recessions.

3a. Lac_v Mal_v⁺
4a. Lac_v Mal_v⁺

Relatively stable; few sectors!
Not unusually stable! 699-3R1
699-4R1

Balanced lethals700
C

March 4, 1950.

- a) Monolite 698-0 (control) and 698-40 (X-ray 40,000) 1:1000 in Peussey and acetate.
effort to induce segregation.
- b. 698B1-7 are 7 stable lac+ from X-rayed H226.
695-92A is a single pure lac+ from UV H226.

Test balanced lethals. 700=[1-7]; 695:1-3. Stocks on
EMBLac, Mal, Xyl, Mtl. Brush EMS Lac.

	lac	Mal	Xyl	Mtl	
1	+ V	-	+, -	++ +, +	{ disqualify as stable diploid!
2	+ V	-	+ -	++	
3	+	+	+	++	
4	+	-	++ shw	++	Only #3 is uniform +; others show
5	+	-	++ sh	+	various changes.
6	+	-	++ sh	+	
7	+	+	=	+	All grow <u>very poorly</u> on EMS Lac.
"8	+	-	=	-	
9	+	-	+	+	
10	+ & shw	-	-	-	

see 693d.

#1 has some colonies clearly almost pure lac+, others lacv!
Lethal lac+: These give predominantly apparently pure + colonies. A very rare colony may appear -. Pick a type + and use as 700-2.

P7: Plate 5 ml growth culture of 700-3 with T6; also K12.
Characteristics of 700-3: 3/8 - Melt + ~~prototrophic~~ (not prototrophic)

700-3 is probably not diploid: a) it gives pure Mal + non-crossing.
b) V_b^R mutants are pure lac +

Search for balanced lethals

700a
A-B

March 5, 1950.

A. B.
Bromulate H226 and H226-XRay 40,000 into ~~+~~ Pernassay and etc.

P5: Plate 1st culture. 3P5 bromulate ca 1:1000 into fresh Pernassay.
10P5 Reisolate. (3rd culture). Also B2.

B1 and B2 on EMBAc < 1/2% Lac+. Pick few + from B1 and B2

All appear to be lac+ = 700B:1-6

A1 ca. 10%+. Pick 40+ and streak out.